Chapter 4. Antimicrobial consumption in animals

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Published in:
DANMAP 2010 - Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark

Publication date:
2011

Document Version
Publisher's PDF, also known as Version of record

DANMAP 2010

DANMAP 2010

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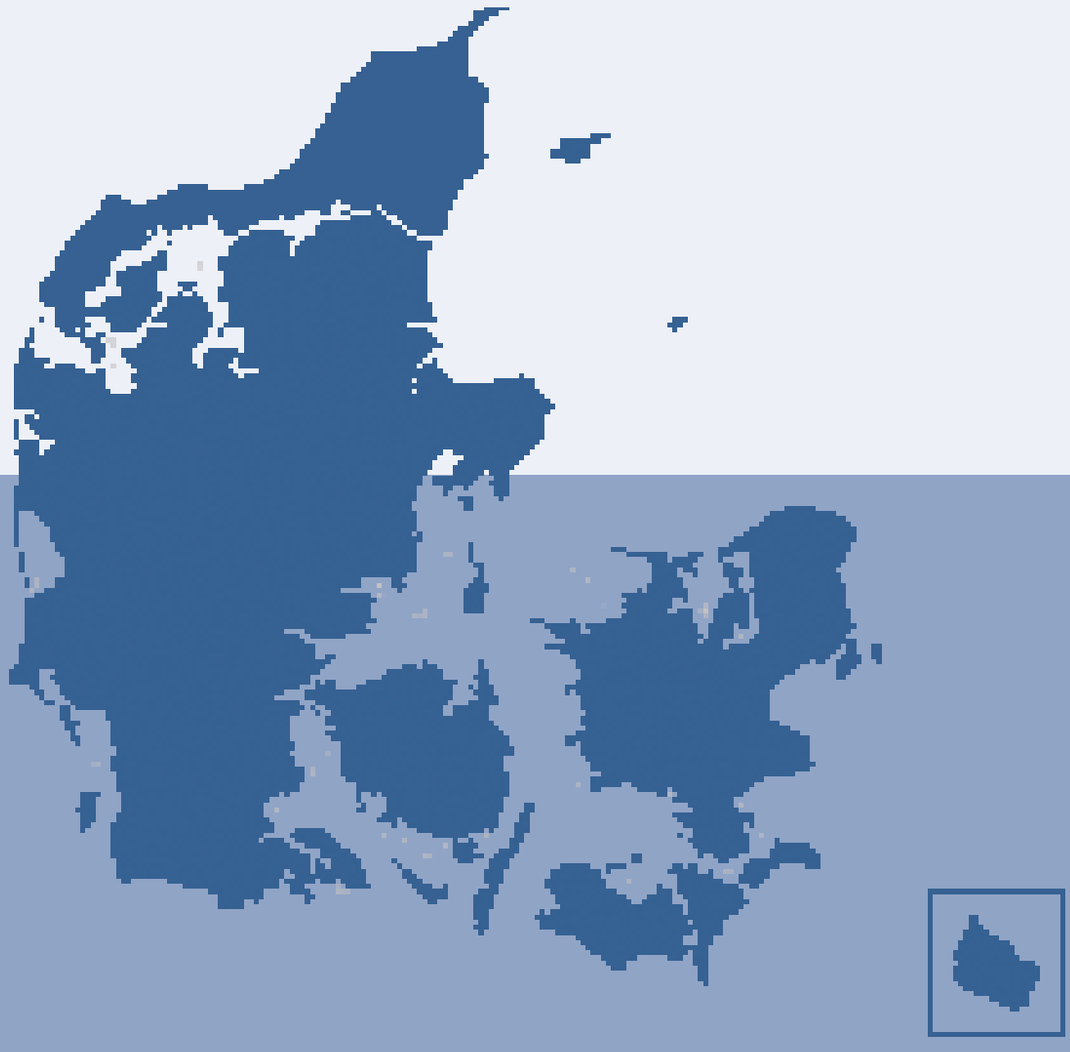

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Printing: Rosendahls-Schultz Grafisk A/S

DANMAP 2010 - August 2011
ISSN 1600-2032

The report is available from
http://www.danmap.org

This report is issued by DANMAP - The Danish Integrated Antimicrobial Resistance Monitoring and Research Programme. It presents the results of monitoring of antimicrobial use and antimicrobial resistance in food animals, food and humans in 2010. The report is produced in collaboration between the National Food Institute, Technical University of Denmark; the National Veterinary Institute, Technical University of Denmark; the Danish Medicines Agency and Statens Serum Institut. The DANMAP programme is funded jointly by the Ministry of Science, Technology and Innovation and the Ministry of Health and Prevention.

DANMAP 2010

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TABLE OF CONTENTS

1. Introduction

1.1 About DANMAP

The Danish Integrated Antimicrobial Resistance Monitoring and Research Programme, DANMAP, was established in 1995 on the initiative of the Danish Ministry of Health and the Danish Ministry of Food, Agriculture and Fisheries, as a coordinated national surveillance and research programme for antimicrobial consumption and antimicrobial resistance in bacteria from animals, food and humans. The participants in the programme are Statens Serum Institut, the National Veterinary Institute, the National Food Institute, and the Danish Medicines Agency. The DANMAP programme is funded jointly by the Ministry of Health and the Ministry of Science, Technology and Innovation.

The objectives of DANMAP are:

- to monitor the consumption of antimicrobial agents for food animals and humans.
- to monitor the occurrence of antimicrobial resistance in bacteria isolated from food animals, food of animal origin and humans.
- to study associations between antimicrobial consumption and antimicrobial resistance.
- to identify routes of transmission and areas for further research studies.

The monitoring of antimicrobial resistance is based on three categories of bacteria: Human and animal pathogens, zoonotic bacteria, and indicator bacteria.

Human and animal pathogens are included because these cause infections and they reflect primarily resistance caused by use of antimicrobial agents in the respective reservoirs. Zoonotic bacteria are included because they can develop resistance in the animal reservoir, which may subsequently compromise treatment effect when causing infection in humans. Indicator bacteria are included due to their ubiquitous nature in animals, food and humans and their ability to readily develop antimicrobial resistance in response to selective pressure in both reservoirs.

This report, DANMAP 2010, describes the annual consumption of antimicrobial agents and the occurrence of resistance in different reservoirs in Denmark in 2010. Results from the monitoring program as well as from selected research projects are presented in overview tables and figures. In the Appendices, detailed tables of antimicrobial consumption in animals and humans and specific MIC distributions are presented, along with a list of abbreviations, explanations of terminology and description of materials and methods. A list of DANMAP publications in the international scientific literature in 2010 is also included.

This DANMAP report is also available at www.danmap.org.

1.2 Acknowledgements

The National Food Institute and the National Veterinary Institute from the Technical University of Denmark would like to thank:

- the meat inspection staff and the company personnel at the slaughter houses for collecting samples from animals at slaughter. Without their careful recording of the animals' farm of origin the results would be less useful.
- the Laboratory of Swine Diseases, Danish Meat Association at Kjellerup for making isolates of animal pathogens available to the programme.
- the Danish Medicines Agency for collecting and transmitting data on veterinary consumption of antimicrobial agents from the pharmacies.
- the staff of the Regional Veterinary and Food Control Authorities for collection of food samples and isolation of bacteria.
- the staff of the Zoonosis Laboratory at the National Food Institute.
- the staff of the research group of Antimicrobial resistance and molecular typing at the National Food Institute.
- the staff of the division of Poultry, Fish and Fur Animals at the National Veterinary Institute.

Statens Serum Institut would like to thank

- the Departments of Clinical Microbiology in the DANRES group - Danish Study Group for Antimicrobial Resistance Surveillance - for providing data on resistance in bacteria from human clinical samples.
- the staff of the Neisseria and Streptococcus Typing Unit at SSI.
- the staff of the Foodborne Pathogens Unit at SSI.
- the staff of the Staphylococcus Laboratory at SSI.
- the staff of the Antimicrobial Resistance Reference Laboratory and Surveillance Unit at SSI.
- Maja Laursen and Jan Poulsen from the Danish Medicines Agency for providing data on consumption of antimicrobials in humans.
- Erik Villadsen from the Danish National Board of Health for providing data on hospital activity.

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2 SAMMENDRAG / SUMMARY

2. Summary

2.1 Sammendrag

Dette er den femtende DANMAP rapport. DANMAP 2010 beskriver det årlige forbrug af antibiotika og forekomsten af resistens i forskellige reservoirer. Den kontinuerlige overvågning af antibiotikaresistens og -forbrug gør det muligt at analysere tendenserne i antibiotikaforbrug og -resistens over tid.

DANMAP præsenterer antibiotikaforbrug til mennesker og dyr på årsbasis. Lægemiddelstyrelsen har overvåget forbruget af receptordineret medicin på patientniveau siden begyndelsen af 1990erne. Siden 2001 er al anvendelse af receptordineret medicin til dyr registreret på dyreart, aldersgruppe og besætningsniveau i VetStat databasen på Veterinærinstituttet, Danmarks Tekniske Universitet.

Antibiotikaforbrug til dyr

I 2010 var antibiotikaforbruget til dyr i Danmark på 126,9 ton, hvilket repræsenterer en 2,1 % reduktion i forhold til 2009. Faldet skyldtes hovedsagligt et mindre forbrug til svin. Størstedelen af det totale antibiotikaforbrug kan henføres til svineproduktionen (79 %), mens en mindre andel kan tilskrives kvæg- (12 %) og fjerkræproduktionen (0,7 %).

Svin: For første gang siden 2002 er der sket et fald i antibiotikaforbruget til svin. Målt i antal antibiotika doser per svin produceret, blev forbruget i 2010 reduceret med 5 % (korrigeret for eksport af 30 kg grise) sammenlignet med 2009, men var fortsat højere end forbruget i 2008. Antibiotika forbruget pr. svin er steget med 39 % over de sidste 10 år.

Faldet i 2010 skete især i forbruget af tetracykliner (5 %), med der var også et reduceret forbrug af makrolider (2 %), aminoglykosider (16 %), lincosamid/spectinomycin (7 %) og cefalosporiner (48 %). Tetracykliner, makrolider og pleuromutiliner, som primært bruges til flok-medicinering i foder eller drikkevand, var fortsat de mest almindelige antibiotika brugt til svin. Faldet i det totale forbrug af antibiotika til svin var for størstedelen forbundet med et 11 % fald i ordinering af antibiotika til fravænningsgrise med tarminfektioner. Ordinationer til so-besætninger (inkl. smågrise) med tarminfektioner faldt med 22 %, svarende til et fald på 3 % per so-år.

Faldet i det totale antibiotikaforbrug relaterer sig kun til det andet halvår af 2010. Forbruget steg reelt med 8 % i de første seks måneder i forhold til samme periode i 2009. I juli 2010 modtog 20 % af de danske svineproducenter, som havde det højeste forbrug af antibiotika, et informationsbrev, der beskrev den nye 'Gult kort' ordning. I samme måned indførte industrien et frivilligt stop for brugen af cefalosporiner til svin. Samlet er dette en sandsynlig forklaring på den 13 % reduktion i antibiotikaforbruget til svin, der blev observeret i anden halvdel af 2010 sammenlignet med samme periode året før.

Kvæg: Antibiotikaforbruget til kvæg var 14,6 ton i 2010, og har været relativt stabilt på omkring 14 til 15 ton siden 2005. I denne periode er andelen af smalspektrede (beta-lactamase følsomme) penicilliner til køer steget fra 48 % til 59 % af doser til systemisk behandling, mens makrolider faldt fra 11 % til 3 %, hvilket er i overensstemmelse med de officielle anbefalinger. Også til kalve faldt forbruget af makrolider fra 35 % af forbruget i 2009 til 24 % af forbruget i 2010, mens forbruget af tetracykliner steg fra 26 % til 30 % af forbruget, hvormed tetracykliner igen blev de mest anvendte antibiotika til kalve. Der var meget få ordinationer af fluorokinoloner i 2010 (1 kg i alt). Forbruget af tredje og fjerde generations cefalosporiner til intramammær og systemisk behandling faldt med hhv. 29 % og 17 % i forhold til 2009.

Fjerkræ: Det totale antibiotikaforbrug til fjerkræ faldt med 18 % i 2010 i forhold til 2009 (fra 1.070 kg til 879 kg), men niveauet ligger stadig højere end i perioden fra 2001 til 2008. Antibiotikaforbruget i kyllingeproduktionen er generelt lavt, og sygdomsudbrud hos nogle få producenter kan medføre betydelige fluktuationer i det totale antibiotikaforbrug. I 2009 var der sygdomsproblemer i adskillige fjerkræflokke, hvilket medførte et relativt højt forbrug. Disse problemer synes løst i æglæggere samt i opdræt til slagtekyllinger. I slagtekyllingeflokkene var antibiotikaforbruget i 2010 fortsat relativt højt i forhold til perioden 2001–2008. Forbruget på 0,14 ADD_{kg} pr. kg kyllingekød produceret er imidlertid fortsat lavt i forhold til andre dyrearter, og også meget lavt i forhold til forbruget i kyllingeproduktionen i ikke-skandinaviske lande.

Antibiotikaforbruget i kalkunproduktionen varierer også markant fra år til år. Sygdomsproblemer medførte i 2009, at forbruget var højt sammenlignet med de forrige år. En vaccinationskampagne mod *Pasteurella multocida* synes at have reduceret sygeligheden, og har medført at antibiotikaforbruget i 2010 var på det laveste niveau siden 2005 (0,62 ADD_{kg} pr. kg kød produceret).

I 2010 blev fluorokinoloner ikke ordineret til kalkun-, æglægger- og i slagtekyllingeproduktionen. Forbruget af fluorokinoloner i fjerkræproduktionen har været faldende siden 2006, hvor fluorokinoloner udgjorde 7 % af det totale forbrug for både kyllinger/høns og kalkuner.

Akvakultur: Det totale forbrug i 2010 var på 3.060 kg, en 7 % reduktion i forhold til 2009. Faldet skyldes primært et skifte i præparatvalg. Havbrug har generelt et højt antibiotikaforbrug pr. kg fisk produceret, sammenlignet med andre dyregrupper, men forbruget har været faldende siden 2006, hvor forbruget toppede med 13 ADD_{kg} pr. kg fisk produceret, i forbindelse med usædvanligt varm sommer. Antibiotikaforbruget i akvakultur er kraftigt påvirket af vandtemperaturen. Faldet er desuden relateret til en markant forbedret vaccinationsstrategi i samme periode (2006-2010), hvor forbruget er faldet med 51% i havbruget, til 9 ADD_{kg} pr. kg fisk produceret. Forbruget i ferskvandsfisk ligger mere stabilt omkring 2 ADD_{kg} pr. kg fisk produceret. Sulfonamid kombineret med trimethoprim samt kinoloner (oxolinsyre) var de mest anvendte antibiotika til fisk.

Kæledyr og heste: Antibiotikaforbruget til kæledyr og heste var i 2010 på 3 tons. Forbruget er estimeret ud fra ordinationer til disse dyrearter samt salg af præparater til smådyrs- og hestepraksis. For 2010 blev det totale forbrug af fluorokinoloner estimeret til 14 kg, hvoraf størstedelen (>13 kg) blev brugt til kæledyr. Dette svarer til 72 % af det totale veterinære forbrug af fluorokinoloner. Amoxicillin kombineret med clavulansyre var det mest brugte antibiotika til kæledyr (539 kg), hvilket udgør en stigning på 3% sammenlignet med 2009.
I 2010 var forbruget af cefalosporiner til kæledyr på 320 kg. Dette var primært 1. generations cefalosporiner til oral behandling, men forbruget af 3. generations og 4. generations cefalosporiner var omkring 3 kg, svarende til 1,8 % af det totale veterinære forbrug.

Antibiotikaforbrug til mennesker

Primærsektor og hospitalssektor: Det totale forbrug af antibiotika til systemisk brug i mennesker (primærsektor og hospitalssektor) steg med 5 %: fra 17,89 DDD pr. 1000 indbyggere pr. dag (DID) i 2009 til 18,84 DID i 2010. Hospitalsforbruget udgjorde 10 % af det totale forbrug. Stigningen i forbruget blev kun observeret i primærsektoren. Siden 2001 er det totale forbrug steget med 4,54 DID (32 %).

Primærsektor: I 2010 steg det totale antibiotikaforbrug (J01) i primærsektoren med 6 % til 16,93 DID sammenlignet med 15,95 DID i 2009. Det er det højeste forbrug, der er målt i DANMAPs historie. Beta-laktamase følsomme penicilliner repræsenterede den største gruppe af antibiotika i 2010 (31 %) og penicilliner (J01C) udgjorde 62 % af det totale forbrug i 2010.
Forbruget af bredspektrede antibiotika var 6,48 DID i 2010; en stigning på 0,53 DID i forhold til 2009. Forbruget af antibiotika steg for alle grupper af antibiotika med undtagelse af sulfonamider.

Der kan være flere forskellige forklaringer på det stigende forbrug: 1) en stigning i antallet af behandlede patienter; 2) udbrud med *Mycoplasma pneumoniae* i anden halvdel af 2010, som medførte et øget forbrug af beta-laktamase sensitive penicilliner til empirisk behandling af nedre luftvejsinfektioner og makrolider til behandling af bekræftet *M. pneumoniae* pneumoni – ifølge de nationale retningslinjer; og 3) et øget forbrug af "kombinationspenicilliner", sandsynligvis som følge af bedre opslutning til de ændringer i behandlingsvejledningerne for patienter med kronisk obstruktive lungelidelser, der kom for få år siden.

Siden 2001 er det totale forbrug (J01) i praksis steget med 32 %; DDD er den forbrugsindikator, som er steget mest, men også antallet af behandlede patienter og antal pakninger er steget i samme periode.

Hospitalssektor: Det totale forbrug af antibiotika i hele hospitalssektoren (rehabiliteringscentre, hospice, privat-, psykiatriske-, specialiserede- og somatiske hospitaler) lå på 1,91 DID i 2010 (svarende til forbruget i 2009). Siden 2001 er det totale forbrug steget med 0,46 DID (31 %). Bredspektrede antibiotika udgjorde 67 % af det totale forbrug på hospitalerne i 2010 ligesom i 2009.

Somatiske hospitaler: Det totale antibiotikaforbrug steg med 3 % opgjort i DDD pr. 100 sengedage (DBD) (fra 85,03 DBD i 2009 til 87,72 DBD i 2010), mens det faldt med 4 % opgjort i DDD pr. 100 indlæggelser (DAD) i forhold til 2009 (fra 297,36 DAD til 284,89 DAD). Antallet af DDD i 2010 var det samme som i 2009, mens antallet af indlæggelser steg og antallet af sengedage faldt i forhold til 2009.

For tre grupper af antibiotika steg forbruget fra 2009 til 2010: kombinationspenicilliner steg med1,48 DBD (26 %); carbapenemer steg med 0,88 DBD (28 %) og kombinationen af sulfonamider/trimethoprim steg med 0,76 DBD (34 %). Forbruget faldt fra 2009 til 2010 for følgende stofgrupper: penicilliner med udvidet spektrum faldt med 0,76 DBD (5 %); beta-laktamase følsomme penicilliner faldt med 0,41 DBD (4 %); 3. generations cefalosporiner faldt med 0,16 DBD (11 %); og fluorkinoloner faldt med 0,27 DBD (2 %). I 2010 udgjorde cefalosporiner 20 % af det totale forbrug på de somatiske hospitaler. Penicilliner med udvidet spektrum (17 %), fluorkinoloner (12 %) og beta-laktamase følsomme penicilliner (11 %) var andre af de mest anvendte antibiotika i 2010.

Over de sidste 10 år er det totale forbrug steget med 39,40 DBD (82 %) eller 35,13 DAD (14 %) afhængig af nævneren. Det er vigtigt at påpege, at antallet af udskrivelser er steget med 18 % i løbet af de sidste 10 år, mens antallet af sengedage er faldet med 26 % som følge af ændringer i hospitalssektoren.

Det totale antibiotikaforbrug til dyr faldt i 2010, og for første gang siden 2002 faldt forbruget til svin. Derimod steg det humane antibiotikaforbrug til det højeste niveau siden starten af DANMAP programmet i 1995. Stigningen i forbruget blev kun observeret i primærsektoren, og kunne delvist forklares med en stigning i antallet af behandlede patienter og et udbrud med *Mycoplasma pneumoniae* i anden halvdel af 2010.

Resistens i zoonotiske bakterier

Zoonotiske bakterier som *Salmonella* og *Campylobacter* er sygdomsfremkaldende bakterier, der kan overføres fra dyr til mennesker, enten via direkte kontakt med dyr eller via kontaminerede fødevarer.

De højeste niveauer af resistens blev fundet i importeret kalkunkød, hvor ingen af de isolerede *S.* Typhimurium isolater var fuldt følsomme overfor alle 16 antibiotika inkluderet i testpanelet, og 93 % af isolaterne var multiresistente. Desuden steg apramycin, gentamicin og streptomycin resistensen signifikant fra 2009 til 2010.

Blandt *Salmonella* Typhimurium isolater fra danske svin blev der fra 2009 til 2010 observeret signifikante stigninger i antibiotikaresistens overfor ampicillin, streptomycin og tetracyklin. Der var ingen signifikante ændringer i resistensforekomsten i dansk svinekød, men tetracyklinresistensen i *S.* Typhimurium isolater fra dansk svinekød (27 %) var signifikant lavere end blandt isolater fra danske svin (47 %). *S.* Typhimurium fra importeret svinekød havde en højere resistensforekomst (for 8 ud

af 16 testede antibiotika) sammenlignet med isolater fra dansk svinekød.

I 2010 blev ingen *S.* Typhimurium isolater fra svin, kvæg, dansk svinekød, importeret svinekød og importeret kylligekød fundet resistente overfor cefalosporiner eller fluorokinoloner. Kun *S.* Typhimurium isolater fra importeret kalkunkød var resistente overfor disse antibiotika. Blandt de humane tilfælde blev der observeret en højere ciprofloxacin resistens hos de rejserelaterede tilfælde (14 %) i forhold til de tilfælde, som havde erhvervet infektionen i Danmark (4 %). Cefalosporin resistens blev kun rapporteret fra rejserelaterede tilfælde (3 %), eller hvor oprindelsen af infektionen var uoplyst (1 %).

Klonal spredning har haft stor indflydelse på udbredelsen af antibiotikaresistens blandt *Salmonella* bakterierne; dette gælder især for *S.* Typhimurium. Siden 2005 har man blandt *S.* Typhimurium isolater fra svin kunnet observere en parallel stigning (14 %) i resistens overfor ampicillin (A), streptomycin (S), sulfonamid (Su) samt tetracyklin (T). Dette resistensmønster (ASSuT) forekommer ofte i fagtyperne DT120 og DT193, som er almindeligt forekommende i svin. En anden almindeligt forekommende klon blandt *S.* Typhimurium fra svin er isolater med resistens overfor ASSuT samt chloramphenicol (C), og dette resistensmønster (ACSSuT) relateres primært til fagtypen DT104. Blandt de humane *S.* Typhimurium isolater blev denne sammenhæng mellem resistensmønstre og fagtyper også observeret.

I de seneste år er der udarbejdet et smittekilderegnskab for *Salmonella*, som angiver de vigtigste fødevarebårne kilder til human salmonellose i Danmark. Denne model blev benyttet til at estimere kilderne til de humane infektioner forårsaget af *S.* Typhimurium med resistens overfor ampicillin, sulfonamid og tetracyklin (ASuT). Modellen estimerede, at dansk svinekød kunne tilskrives et udbruds-relateret ASuT-tilfælde og fem sporadiske ASuT–tilfælde, importeret svinekød 41 ASuT-tilfælde, endvidere blev et ASuT-tilfælde tilskrevet importeret kalkunkød.

Salmonella Enteritidis er relativt sjælden i dansk fjerkræproduktion og kun få isolater var til rådighed fra dansk fjerkræ og fjerkrækød i 2010. Kun isolater fra importeret kyllingekød var resistente overfor nalidixansyre og ciprofloxacin. Humant var resistensen overfor ciprofloxacin signifikant højere i rejseassocierede tilfælde (21 %) end i tilfælde erhvervet i Danmark (8 %).

I 2010 var der ingen signifikante ændringer i resistensforekomsten blandt *Campylobacter jejuni* isolater fra danske slagtekyllinger og kvæg eller blandt *Campylobacter coli* isolater fra svin. Siden 2005 er der sket en svag stigning i forekomsten af tetracyklin resistens blandt *C. jejuni* fra danske slagtekyllinger og kvæg samt i *C. coli* fra danske svin. I samme periode var tetracyklin et af de mest almindelige antibiotika ordineret til svin.

Som i de foregående år indeholdt importeret kyllingekød *C. jejuni* med signifikant højere resistens overfor ciprofloxacin (50 %) sammenlignet med dansk kyllingekød (17 %).

Blandt de humane *C. jejuni* isolater fra tilfælde erhvervet i Danmark, var der i 2010 ingen signifikante ændringer i resistensforekomsten i forhold til 2009. Ciprofloxacin resistensen blandt *C. jejuni* isolater fra infektioner erhvervet herhjemme (25 %) var signifikant lavere end blandt isolater fra rejserelaterede tilfælde (80 %).

Forekomsten af *Clostridium difficile* i svinebesætninger, samt hos kvæg og kyllinger på slagterierne blev for første gang undersøgt i 2010. *C. difficile* blev isoleret fra 15 % af svinebesætningerne, 15 % af kvæget og 3 % of kyllingeflokkene. Alle tre toksin-gener blev påvist i 73 % af svine-isolaterne og 24 % af kvæg-isolaterne, mens et eller to toksin-gener blev påvist i de resterede isolater. Isolater med tre toksin-gener blev PCR ribotypet, og PCR ribotype 078 blev fundet både blandt svine- og kvægisolater. Ribotype 078 forekommer hos mennesker, mens de resterende PCR ribotyper er sjældent eller aldrig fundet i humane isolater i Danmark. Isolaterne blev testet for resistens overfor fem antibiotika, og de fleste isolater var resistente overfor clindamycin (87 %), mens resistens overfor de andre antibiotika var relativ lav.

Fundet af *C. difficile* 078 i svin er forventelig, da denne type er almindelig blandt svin. Men nogle af de andre typer med alle tre toksin-gener samt deletioner i *tcdC* kan muligvis forårsage alvorlig human sygdom. Den potentielle humane risiko ved forekomst af *C. difficile* med *tcdA* og *tcdB* toksin-gener i husdyr bør undersøges nærmere.

Resistensforekomsten i S. Typhimurium fra svin steg i 2010, mens forekomsten i dansk svinekød forblev på samme niveau som i 2009. Resistensforekomsten i S. Typhimurium fra dansk svinekød og i Campylobacter jejuni i dansk kyllingekød var signifikant lavere end i isolater fra det importerede kød. Et tilsvarende mønster blev observeret blandt de humane S. Typhimurium og Campylobacter jejuni infektioner, hvor rejserelaterede tilfælde havde signifikant højere resistensforekomst sammenlignet med tilfælde som havde erhvervet infektionen i Danmark.

Resistens i indikatorbakterier

Indikatorbakterier er inkluderet i overvågningsprogrammet for at give information om de generelle resistensniveauer i sunde og raske husdyr.

Blandt *Enterococcus faecium* isolater fra svin og slagtekyllinger blev der observeret signifikante fald i resistens overfor tetracyklin, penicillin og ampicillin fra 2009 til 2010. Desuden faldt forekomsten af streptomycin resistens i isolater fra svin, og resistens overfor quinupristin/dalfopristin og avilamycin i isolater fra slagtekyllinger. Sammenlignet med importeret kyllingekød var resistensforekomsten signifikant lavere i dansk produceret kyllingekød (for 7 ud af 16 testede antibiotika). Ved brug af selektive opformeringsmetoder blev der påvist vancomycin resistente *E. faecium* i 47 % af slagtekyllingerne. Dette indikerer udbredt forekomst af VRE i lave koncentrationer i en stor del af besætningerne, selv om brug af vækstfremmeren avoparcin har været forbudt i Danmark siden 1995.

I 2010 havde *Enterococcus faecalis* isolater fra slagtekyllinger signifikant lavere resistens overfor tetracyklin,

erythromycin, streptomycin og salinomycin sammenlignet med 2009, mens det blandt isolater fra svin kun var tetracyklin resistensen, som blev reduceret signifikant. Faldet i tetracyklin resistens blandt enterokok isolater fra svin kan være relateret til de registrerede fald i forbruget af tetracyklin.

En undersøgelse viste, at det var den samme klon af 'høj niveau' gentamicin-resistente *E. faecalis* (ST16) som blev påvist i svin, svinekød, raske personer og fra patienter med infektiøs endokardit. Dette indikerer en zoonotisk sammenhæng.

I 2010 var der ingen signifikante ændringer i resistensforekomsten blandt indikator *E. coli* isolater fra svin og slagtekyllinger sammenlignet med 2009. Der var en signifikant stigning i tetracyklin resistensen blandt isolater fra kvæg (fra 2 % til 9 %), formentlig relateret til en 10% stigning i tetracyklinforbruget til kalve i 2010. I 2010 blev der ikke observeret fluorokinolon resistens blandt *E. coli* fra danske svin og kvæg, derimod var 8 % af *E. coli* isolater fra slagtekyllinger fluorokinolon resistente. I 2002 blev brugen af fluorokinoloner til behandling af husdyr begrænset, og forbruget har siden da generelt været lavt. Forekomsten af fluorokinolon resistens i kyllingeproduktionen kan hænge sammen med, at forbruget her er relativt højere end i de andre husdyrgrupper.

Som for *Campylobacter* og enterokokker var resistensforekomsten i *E. coli* isolater fra importeret kyllingekød signifikant højere end i isolater fra dansk kyllingekød (for 13 ud af 16 testede antibiotika), og 60 % af isolaterne fra importeret kyllingekød var multiresistente. Ceftiofur resistens (og dermed ESBL) blev i 2010 observeret for første gang uden brug af selektiv opformering i et *E. coli* isolat fra dansk kyllingekød (1 %). Fluorokinolon resistensen var ti gange højere i importeret kyllingekød (41 %) end i dansk produceret kyllingekød (4 %).

Blandt *E. coli* isolater fra dansk svinekød faldt sulfonamid resistensen signifikant fra 38 % til 19 %, og i 2010 var resistens overfor tetracyklin og sulfonamid signifikant lavere i *E. coli* isolater fra dansk svinekød sammenlignet med isolater fra importeret svinekød. Fluorokinolon resistensen i dansk svinekød var fortsat lav (et isolat) i forhold til 4 % af *E. coli* isolaterne fra importeret svinekød.

Blandt *E. coli* isolater fra kvæg og fra dansk og importeret oksekød var resistensen lav.

ESBL-producerende bakterier er resistente overfor bredspekterede cefalosporiner, der ofte bruges til behandling. Derfor er forekomsten af disse, selv i et lavt niveau, et potentielt alvorligt problem. Ved brug af selektive opformeringsmetoder blev forekomsten af disse bakterier undersøgt i svinebesætninger, hos kvæg og kyllinger på slagterierne samt i kød fra detailforretninger og engroslagre. Den højeste forekomst af ESBL-producerende *E. coli* hos dyrene blev påvist i kyllingeflokke på slagteriet (27 %), på trods af at cefalosporiner ikke har været brugt i den danske kyllingeproduktion de sidste ti år. I kødprøverne blev de højeste forekomster af ESBL-producerende *E. coli* påvist i importeret (50 %) og dansk (8.6 %) kyllingekød. Forekomsten af ESBL-producerende *E. coli* fra importeret kyllingekød var i 2010 signifikant højere end i 2009.

Tilstedeværelsen af de forskellige ESBL-gener afhang af dyrearten. CMY-2 og SHV-2 blev ofte fundet hos slagtekyllinger, mens CTX-M-8 kun blev påvist hos kvæg. Flere af ESBL-generne i *E. coli* fra dyr og kød er tidligere fundet i humane *E. coli* isolater. Slagtekyllinger og kyllingekød synes at være et vigtigt reservoir for ESBL-producerende *E. coli,* også i lande som Danmark, hvor brugen af cefalosporiner i kyllingeproduktionen for længst er ophørt eller aldrig har været brugt.

I Danmark kan slagtekyllinger og kyllingekød være et vigtigt reservoir for ESBL-producerende *E. coli*, selvom cefalosporiner ikke benyttes i kyllingeproduktionen. Der er stadig en lav forekomst af resistens overfor vancomycin og quinupristin/dalfopristin blandt *E. faecium* isolater fra svin, selvom brugen af vækstfremmere har været forbudt i mere end ti år.

Resistens i bakterier fra diagnostiske indsendelser fra mennesker

Rapporteringen af antibiotikaresistens i bakterier fra diagnostiske indsendelser fra mennesker er baseret på frivillig indsendelse af data fra DANRES gruppen, som dækker de klinisk mikrobiologiske afdelinger i Danmark. De eneste undtagelser omfatter methicillin resistente *Staphylococcus aureus* og invasive *Streptococcus pneumoniae*, som er anmeldepligtige. Data vedrørende disse bakterier kommer fra referencelaboratorierne på SSI.

Blandt *E. coli* isolater fra blod var 3. generations cefalosporin resistensforekomsten i 2010 på 7 %, det samme niveau som i 2009. Niveauet var højere end i de andre nordiske lande i 2009. I 2010 steg gentamicin resistensforekomsten til 6 %. Ciprofloxacin resistensen var 14 % i 2010 (min 7 %, max 22 % for de individuelle KMAer), dette niveau var det samme som i 2009. Ingen *E. coli* isolater fra blodinfektioner var carbapenem resistente. I løbet af de seneste 10 år er resistensen overfor cefuroxim, ciprofloxacin og gentamicin steget signifikant. Resistens overfor 3. generations cefalosporiner er rapporteret til DANMAP siden 2008; i denne periode er resistensforekomsten steget.

Blandt *E. coli* isolater fra urin fra hospitaler var 3. generations cefalosporin resistensforekomsten på 5 % i 2010, det samme niveau som i 2009. For de følgende antibiotika var der et lille fald (1 %) i resistensforekomsten: ampicillin (41 %), sulfonamid (35 %), ciprofloxacin (12 %) og cefuroxim (2. generations cefalosporin) (5 %).

Blandt *E. coli* isolater fra urin fra praksis var 3. generations cefalosporin resistensforekomsten på 5 % i 2010, det samme niveau som i 2009. Nalidixansyre resistensen steg fra 14 % i 2009 til 15 % in 2010. Fra 2009 til 2010 var der små fald i resistensforekomsten (1-2 %) for ampicillin (40 %) og sulfonamid (37 %).

Blandt *Klebsiella pneumoniae* isolater fra blod var resistensforekomsten for 3. generations cefalosporiner 9 % (min. 4 %, max 24 %), hvilket er samme niveau som i 2009. Denne resistensforekomst var højere, end hvad der blev rapporteret til EARS-Net for de andre nordiske

lande i 2009 og på samme niveau som i flere sydeuropæiske lande. Forekomsten af 3. generations cefalosporin resistens var signifikant højere i den østlige del af Danmark (14 %) sammenlignet med den vestlige del (6 %). Både fluorkinolon resistensen (ciprofloxacin 11 %, nalidixansyre 17 %) og gentamicin resistensen var højere end i de andre nordiske lande og på samme niveau som i flere sydeuropæiske lande. Fra 2009 til 2010 var der et fald i resistensforekomsten for gentamicin, ciprofloxacin og cefuroxim; dette fald sås mest for *K. pneumoniae* isolater på Sjælland. Dette kunne delvis forklares ved intervention på hospitaler i Københavnsområdet (Tekstboks 8). Ingen *K. pneumoniae* isolater fra blod var carbapenem resistente.

Blandt *Klebsiella pneumoniae* isolater fra urin var forekomsten af resistens for 3. generations cefalosporiner 12 % i isolater fra hospitaler og 7 % i isolater fra primærsektoren, dette var på samme niveau som i 2009. Både for isolaterne fra hospital- og praksis-urinprøverne var forekomsten af resistens for 3. generations cefalosporiner og ciprofloxacin signifikant højere i den østlige del af Danmark (Sjælland) end i den vestlige del (Fyn og Jylland). Der var et signifikant fald i forekomsten af fluorkinolon resistens i *K. pneumoniae* urinisolater fra hospitalerne fra 2009 til 2010 (i 2010: ciprofloxacin 14 %, nalidixansyre 20 %). Sulfonamid resistensen steg til 29 % blandt urinisolater fra hospitalerne og til 34 % blandt isolaterne fra praksis.

Carbapenem (meropenem) resistens var til stede i *K. pneumoniae* urinisolaterne fra både hospitals- og praksissektor. Et af de carbapenem resistente isolater producerede det nye carbapenemase enzym New Delhi Metallo-β-lactamase 1 (NDM-1). Dette isolat var resistent overfor alle testede antibiotika undtagen tigecyclin og colistin. Forekomsten af carbapenem resistens i *K. pneumoniae* er ikke anmeldepligtig; derfor kunne der ikke beregnes en frekvens for carbapenem resistens.

ESBL-producerende *E. coli* og *K. pneumoniae* er ikke anmeldepligtige i Danmark, og de var kun rapporteret til DANMAP fra få KMAer; det var derfor ikke muligt at beregne forekomsten.

Resistensforekomsten i *Pseudomonas aeruginosa* isolater fra blod var lav for alle de testede antibiotika.

I 2010 var penicillin og erythromycin resistensforekomsten stadig lav blandt *Streptococcus pneumoniae* og gruppe A, B, C og G streptokokker.

Forekomsten af ampicillin resistens i *Enterococcus faecium* isolater fra blod steg i 2010 til 92 %. Forekomsten af vancomycin resistens var 1.8 % hos *E. faecium* og mindre end 1 % i *E. faecalis* blodisolater. I 2010 var der et udbrud med vancomycin resistente (*vanA*) *E. faecium* på Aarhus Universitetshospital. Dette udbrud er stadig ved at blive undersøgt. Høj niveau gentamicin resistens (HLGR) fra blodinfektioner blev kun testet på én afdeling for klinisk mikrobiologi. Her var 36 % af de testede *E. faecalis* isolater HLGR og 74 % af de testede *E. faecium* isolater HLGR.

I 2010 blev der indrapporteret 1.418 tilfælde af *Staphylococcus aureus* bakteriæmi svarende til en incidens på 24.6 pr. 100.000 indbyggere (uændret fra 2009). I alt 20 (1.4 %) var forårsaget af methicillin resistente *S. aureus* (MRSA). Dette er på samme niveau som i 2009 og er fortsat blandt de laveste incidenser observeret i Europa. Frekvensen af resistens overfor fusidinsyre og norfloxacin steg, mens frekvensen af resistens overfor øvrige antibiotika lå på samme niveau som de foregående år.

Antallet af nye tilfælde af MRSA var i 2010 1.097 sammenlignet med 817 i 2009. Antallet var det højeste i mere end 25 år. Stigningen blev set både blandt tilfælde erhvervet i udlandet (247 vs. 156) og tilfælde erhvervet i Danmark (852 vs. 661). For tilfælde i Danmark skyldes dette specielt flere tilfælde i gruppen "samfundserhvervet, med rapporteret kontakt til hospital/plejehjem indenfor de sidste 12 måneder" (169 vs. 81). Hos 129 af disse var der dog ingen kendt MRSA eksponering; stigningen i denne gruppe kan således skyldes bedre rapportering af hospitals-/plejehjemskontakt. Antallet af hospitalserhvervede tilfælde er fortsat lavt og på samme niveau som i 2009 (62 vs. 53).
For samfundserhvervede tilfælde uden kendt hospitals/plejehjemskontakt er der set en signifikant ændring, således at der i 2010 er betydelig flere, der har rapporteret kendt eksponering for MRSA. Dette gælder både for patienter med infektioner samt personer, der er bærere af MRSA (screeningsprøver).

Der blev i 2010 set en signifikant stigning i antallet af humane MRSA af typen CC398, der har relation til svin; fra 40 tilfælde i 2009 til 109 i 2010. I 15 af disse tilfælde har personerne ikke haft direkte kontakt til svin eller bor i husstand med én, der har direkte kontakt til svin; dette kan betyde, at MRSA CC398 er begyndt at adaptere sig, således at den lettere smitter fra menneske til menneske. Hovedparten af disse 15 personer bor i nærområde til andre personer med CC398, eller hvor der er konstateret MRSA CC398 i svin. Der er fortsat ingen tegn til spredning til egentlige byområder, og der er således fortsat ingen tegn på, at MRSA CC398 spredes via kød.

I 2010 blev erkendt et nyt gen, der koder for methicillin resistens, kaldet *mecAlga251*. Disse stammer var negative med hidtidige detektionsmetoder. I 2010 var der i alt 21 personer smittet med denne type. Undersøgelse af tidligere års stammer har vist, at disse har spredt sig i Danmark siden 2004.

Forekomsten af 3. generations cefalosporin resistente *E. coli* og *K. pneumoniae* fra blod- og urinvejs-infektioner var på samme niveau som i 2009. Forekomsten af resistens for tredje generations cefalosporiner og ciprofloxacin var højere i *K. pneumoniae* isolater fra Sjælland sammenlignet med forekomsten på Fyn og i Jylland. Et interventionsstudium på Bispebjerg Hospital viste, at det er muligt at nedbringe antallet af resistente *K. pneumoniae* isolater.
De fleste *E. faecium* isolater var ampicillinresistente. Resistensforekomsten var stadig lav hos *P. aeruginosa* og streptokokker.
Antallet af hospitalserhvervede MRSA var uændret, mens stigningen i MRSA infektioner generelt skyldes en spredning i samfundet udenfor hospitalerne. Der var en stigning i antallet af humane MRSA CC398, en type som er associeret med kontakt til svin.

2.2 Summary

This is the 15th DANMAP report. DANMAP 2010 describes the annual consumption of antimicrobial agents and the occurrence of resistance in different reservoirs. The continuous monitoring of antimicrobial resistance and consumption makes it possible to analyse trends over time.

DANMAP presents the use of antimicrobial agents in humans and animals. In humans, the use of prescription medicines has been monitored by the Danish Medicines Agency at the level of the individual patient since the early 1990s. In animals, data on all medicines prescribed by veterinarians for use in animals have been registered at farm and species level by the VetStat program at the Veterinary Institute (Technical University of Denmark) since 2001.

Antimicrobial consumption in animals

In 2010, the total veterinary consumption of antimicrobial agents amounted to 126.9 tonnes, representing a 2.1% decrease relative to 2009, which was attributed to a decrease in consumption in pigs. The antimicrobial consumption in pigs, cattle and poultry comprised 79%, 12%, and 0.7% of the total veterinary consumption, respectively.

Pigs: For the first time since 2002, the total antimicrobial consumption in pigs decreased. The decrease in consumption was 5% measured in doses per pig produced (adjusted for export of pigs around 30 kg) compared with the 2009 level, but remained above the 2008 level (by 7%). Over the past decade, the consumption per pig produced has increased by 39% (2001–2010).

The decrease in 2010 was mainly in use of tetracyclines with a reduction of 100.5 tonnes, representing a 5% decrease per pig produced. Also the use of macrolides (2%), aminoglycosides (16%), lincosamides/spectinomycin (7%) and cephalosporins (48%) was reduced. Tetracyclines, macrolides and pleuromutilins mainly used for oral therapy, continued to be the most commonly used antimicrobial agents in pigs throughout 2001–2010.

The overall decrease in consumption was mainly associated with an 11% decrease in prescription for weaning pigs primarily for gastrointestinal infections, but also decreasing prescription for sow herds was observed. The consumption in sow herds (including piglets) decreased by 3% per sow-year, related to a 22% decrease in prescription for gastrointestinal disease.

The decrease in consumption in 2010 was entirely related to the second half of the year, while in the first half of the year, the consumption increased by 8% compared to the same period in 2009. The decrease in use of cephalosporins was related to a voluntary ban by the industry enforced in July 2010. The same month, the "yellow card" intervention was announced with an information letter to part of the pig farmers, representing the farms with the 20% highest consumption per pig (Textbox 2). This is a likely explanation for the 13% reduction in the second half year compared with the same period in 2009.

Cattle: In 2010, approximately 14.6 tonnes of antimicrobial agents were prescribed for cattle; overall, the consumption has been stable since 2005, with a small increase in 2009. Since 2005, the proportional use of beta-lactamase sensitive penicillins for cows has been continuously increasing from 48% to 59%, while use of macrolides has decreased from 11% to 3%, in accordance with the official guidelines. In calves, the use of macrolides decreased from 35% in 2009 to 24% in 2010, while tetracyclines increased from 26% to 30% of the total consumption, becoming the major drug of choice as before 2006. In 2006–2009, macrolides were the major drug of choice; the reduction in use of macrolides for calves from 35% in 2009 to 24% in 2010, was in accordance with the official guidelines.

The use of fluoroquinolones in cattle was only one kg. The use of 3rd and 4th generation cephalosporins decreased both for systemic and intramammary use, by 17% and 29% respectively, as compared to 2009. Over the past decade, the highest consumption of 3rd and 4th generation cephalosporins for cattle was in 2008 (a total of 92 kg).

Poultry: The consumption of antimicrobial agents in poultry decreased by 18% to 879 kg in 2010 compared with 2009, but was higher than the levels in previous years, 2001–2008.

The antimicrobial consumption in domestic fowl (*Gallus gallus*) is generally at a very low level. Therefore, disease outbreaks in a few farms affect importantly the national consumption in poultry causing considerable fluctuations. In 2009, increasing disease problems caused a steep increase in consumption [DANMAP 2009]. These problems appeared to be under control in 2010 for the breeding and rearing for layer production, and rearing for broiler production.

For broilers, an additional increase was observed in 2010, mainly in the prescription of amoxicillin. The consumption per broiler produced (including breeding and rearing) was unchanged at 0.14 ADD_{kg} in 2010 compared with 2009, but this was more than double of the consumption during 2001–2008.

The antimicrobial consumption in turkeys also fluctuates significantly between years. The consumption was very high in 2009 compared with previous years but a vaccination campaign (*Pasteurella multocida*) seemed to be successful in the combat of the problems, causing a decrease in antimicrobial consumption to the lowest level since 2005.

In 2010, fluoroquinolones were used neither in the turkey production nor in *Gallus gallus*; the use of fluoroquinolones has been decreasing since 2006 when fluoroquinolones comprised 7% of the consumption both in *Gallus gallus* and in turkey production.

Aquaculture: The antimicrobial consumption in aquaculture decreased by 7% to 3,060 kg in 2010, continuing the decrease observed in 2009. This was mainly due to a change in choice of antimicrobial agent towards oxolinic acid. The consumption is generally high in salt water aquaculture and peaked in 2006 reaching 13 ADD_{kg}/kg fish produced, due to unusually high summer temperatures. Since then the consumption has decreased by 51% to 9 ADD_{kg}/kg fish produced, partly because of variation i water temperature, partly due to a gradual improvement of vaccination strategies. The consumption in fresh water is more stabile around 2 ADD_{kg}/kg fish produced. In previous years the major class of antimicrobial was sulfonamide/trimethoprim, followed by quinolones (oxolinic acid).

Companion animals: The consumption of antimicrobial agents in companion animals (pet animals and horses) was around 3 tonnes, estimated from the prescription for these species and sales for companion animal practices. The use of fluoroquinolones in companion animals was estimated to 14 kg in 2010 (>13 kg for pet animals), corresponding to 72% of the total veterinary consumption of fluoroquinolones. The major antimicrobial agent used in pet animals was amoxicillin in combination with clavulanic acid (539 kg), representing an increase of 3% compared with 2009. Other agents frequently used in pet animals were cephalosporins (estimated 320 kg), mainly 1st generation for oral use. In pet animals, the consumption of 3rd and 4th generation cephalosporin was an estimated 3 kg, corresponding to 1.8% of the total veterinary consumption of these antimicrobial agents.

Antimicrobial consumption in humans

Primary health care and hospital care: The total consumption of antibacterial agents for systemic use (primary health care and hospital care) increased by 5%: from 17.89 DDDs per 1,000 inhabitants per day (DID) in 2009 to 18.84 DID in 2010. Hospital care contributed 10% of the total consumption. The increase was noticed in primary health care only. Since 2001, the total consumption of antibacterial agents has increased by 4.54 DID (32%).

Primary health care: The consumption of antibacterial agents for systemic use (J01) in primary health care increased by 6% to 16.93 DID as compared with 15.95 DID in 2009. This is the highest level of consumption measured in the history of DANMAP. Beta-lactamase sensitive penicillins represented the largest therapeutic group of antibacterial agents consumed (31%), and penicillins (J01C) accounted for 62% of the total consumption in 2010. Consumption of broad-spectrum agents represented 6.48 DID in 2010, increasing by 0.53 DID (9%) compared with 2009. Consumption of all but one therapeutic group (short-acting sulfonamides) increased. Possible explanations for the increased consumption include: 1) an increased number of patients treated; 2) an outbreak of *Mycoplasma pneumoniae* in the second half of 2010 with increased consumption of beta-lactamase sensitive penicillins as empirical treatment of lower respiratory tract infection and macrolides as treatment of confirmed *M. pneumoniae pneumonia* - according to national guidelines; and 3) an increased consumption of 'combination penicillins' presumably as a result of better adherence to the changes in the treatment guidelines for patients with chronic obstructive lung diseases that were introduced a few years ago.

Total antibacterial consumption (J01) in primary health care has increased by 32% since 2001, and DDD seems to be the indicator that has increased the most. On a treated-patient-level both the number of DDDs per treated patient and DDDs per prescribed package has increased since 2001.

Hospital care: Total consumption (J01) in Danish hospital care (rehabilitation centres, hospices, private-, psychiatric-, specialised-, and somatic hospitals) added up to 1.91 DID in 2010; similar to that of 2009. Since 2001, the consumption has increased by 0.46 DID (31%). Broad-spectrum agents represented 67% of the total consumption, as in 2009.

Somatic hospitals: The total consumption (J01) expressed in DDDs per 100 occupied bed-days (DBD) increased by 3% (from 85.03 DBD in 2009 to 87.72 DBD in 2010). When expressed as the number of DDDs per 100 admissions (DAD) it decreased from 297.36 DAD to 284.89 DAD (4%). These figures are based on almost equal numbers of DDD, but less occupied bed-days and more admissions in 2010 compared with 2009.

In three therapeutic groups, consumption increased from 2009 to 2010 when expressed as DBD: 'combination penicillins' increased by 1.48 DBD (26%); carbapenems increased by 0.88 DBD (28%) and combinations of sulfonamides and trimethoprim increased by 0.76 DBD (34%). Consumption decreased in other therapeutic groups from 2009 to 2010: penicillins with extended spectrum with a decrease of 0.76 DBD (5%); beta-lactamase sensitive penicillins with a decrease of 0.41 DBD (4%); 3. generation cephalosporins with a decrease of 0.16 DBD (11%); and fluoroquinolones with a decrease of 0.27 DBD (2%). In 2010, cephalosporins accounted for 20% of the total consumption in somatic hospitals. Penicillins with extended spectrum (17%), fluoroquinolones (12%) and beta-lactamase sensitive penicillins (11%) were the other top four contributing therapeutic groups in 2010.

Over the last decade (2001–2010), somatic hospital consumption has increased by 39.40 DBD (82%) or by 35.13 DAD (14%) depending on the denominator. It is imperative to exemplify that the number of admissions has increased by 18% during the last decade and the number of bed-days has decreased by 26% as a consequence of changes in hospitalization patterns.

Overall, the total antimicrobial consumption in animals decreased during 2010, and for the first time since 2002, the consumption in pigs decreased. In contrast, the human consumption increased to the highest level seen since the start of the DANMAP programme in 1995. The increased consumption was observed in primary health care, and could partly be explained by an outbreak of *Mycoplasma pneumoniae* and increased number of treated patients during 2010.

Resistance in zoonotic bacteria

Zoonoses such as salmonellosis or campylobacteriosis are infections and diseases that are transmissible between animals and humans, either via direct contact or indirectly via contaminated food. Data on antimicrobial resistance originate from the DANMAP programme as well as national surveillance and control programmes for *Salmonella* and *Campylobacter.*

Among the *Salmonella* Typhimurium isolates from Danish pigs, a significant increase in resistance to ampicillin, streptomycin and tetracycline was observed from 2009 to 2010. When comparing the resistance in Danish pork (27%) to resistance in Danish pigs (47%), a significantly higher occurrence of resistance to tetracycline was found in the animals. *S.* Typhimurium isolates from imported pork had a significantly higher occurrence of resistance to five of the 16 tested antimicrobial agents than *S.* Typhimurium isolates from Danish pork.

The highest level of resistance was observed in imported turkey meat, where none of the *S.* Typhimurium isolates were fully sensitive, whereas 93% were found to be multi-resistant. In addition, a significant increase in resistance was seen for apramycin, gentamicin and streptomycin resistance in 2010 compared to 2009.

In 2010, no animal isolates of *S.* Typhimurium or *S.* Enteritidis were found resistant to cephalosporins, ciprofloxacin or nalidixic acid. Only *S.* Typhimurium isolates from imported turkey meat were found resistant to these three antimicrobial agents. Among the human cases, a higher level of ciprofloxacin resistance was observed in the travel-associated infections (14%) when compared with the domestically acquired infections (4%), and resistance to cephalosporins was only reported among cases that had travelled abroad (3%) or where the origin of the infection was unknown (1%).

Clonal dissemination plays an important role in the spread of antimicrobial resistant *Salmonella* spp., particularly within *S.* Typhimurium. Since 2005, there has been a parallel increase (14%) in pig isolates resistant to ampicillin, streptomycin, sulfonamide and tetracycline (ASSuT), a resistance pattern often observed in the phage types DT120 and DT193. Among the pig isolates resistant to ampicillin, chloramphenicol, streptomycin, sulfonamide and tetracycline (ACSSuT), the majority were phage type DT104. Among the human *S.* Typhimurium cases, the same correlation between resistance pattern and phage types was observed.

A source attribution model is routinely applied to estimate the contribution of the major animal-food sources to human *Salmonella* infections in Denmark. This model was used to estimate the number of domestically acquired human cases caused by *S.* Typhimurium isolates resistant to ampicillin, sulfonamide and tetracycline (ASuT). Overall, one outbreak-related and five sporadic ASuT cases were attributed to Danish pork, 41 ASuT cases to imported pork and one ASuT case to imported turkey meat.

Among *Salmonella* Enteritidis isolates from human cases, a higher level of ciprofloxacin resistance was observed in the travel-associated infections (21%) and cases of unknown origin (21%) when compared with the domestic sporadic cases (8%).

From 2009 to 2010, no significant changes in resistance were observed among isolates of *Campylobacter jejuni* from Danish broilers and Danish cattle nor for *Campylobacter coli* isolates from pigs. In general, a slightly increasing trend has been observed in the occurrence of resistance towards tetracycline in *C. jejuni* from Danish broilers and Danish cattle, as well as for *C. coli* from Danish pigs since 2005. During the same period, tetracyclines have been the most or second most frequently used group of antimicrobial agents for these animal species. As in previous years, the level of ciprofloxacin resistance in *C. jejuni* was significantly higher among isolates from imported broiler meat (50%) when compared with isolates from Danish broiler meat (17%).

From 2009 to 2010, no significant changes in resistance were observed in *C. jejuni* isolates from human campylobacteriosis cases acquired domestically. However, *C. jejuni* isolates from cases associated with a history of travel have had significantly higher level of ciprofloxacin resistance (80%) compared to domestically acquired cases (25%).

Pig farms as well as cattle and broilers at slaughter were investigated for the occurrence of *Clostridium difficile* for the first time. Fifteen percent of the pig farms, 15% of cattle and 3% of the broiler flocks were positive for *C. difficile.* Isolates with up to three toxin genes were found with the highest occurrence among isolates from pig farms (73%). The isolates with three toxin genes present were ribotyped, and PCR ribotype 078 commonly found among pigs was found among pig farm isolates and cattle isolates. The rest belonged to PCR ribotypes rarely or not previously found in humans in Denmark. The isolates were tested to five antimicrobial agents and most isolates were resistant to clindamycin (87%); resistance was low to the other antimicrobial agents tested.

Findings of *C. difficile* 078 in pigs are not surprising since this type is known to be common among pigs. But other types may also have a potential to cause severe disease in humans as types with all three toxin genes and deletion in *tcdC* were found. Moreover, the importance of *C. difficile* with *tcdA* and *tcdB* in animals should be further investigated.

The level of resistance in *S.* Typhimurium from pigs increased in 2010, whereas the level in Danish pork remained the same. The level of resistance in *S.* Typhimurium from Danish pork and *Campylobacter jejuni* from Danish broiler meat was significantly lower than in isolates from imported meat. A similar pattern was observed among the human *S.* Typhimurium and *Campylobacter jejuni* cases, where cases associated with a history of travel had significantly higher levels of resistance compared to domestically acquired cases.

Resistance in indicator bacteria

Indicator bacteria are included in the DANMAP programme to provide information about the general levels of resistance in healthy production animals and meat.

Enterococcus faecium isolates from both pigs and broilers showed a significant decrease in the occurrence of resistance to tetracycline, penicillin and ampicillin from 2009 to 2010. In addition, a decrease in the occurrence of streptomycin resistance was seen in isolates from pigs, and the occurrence of quinupristin/dalfopristin and avilamycin resistance in isolates from broilers also decreased. When comparing *E. faecium* isolates from Danish and imported broiler meat, a significantly higher occurrence of resistance to seven different antimicrobial agents was found among isolates from imported broiler meat. Using a selective enrichment method, vancomycin resistant *E. faecium* was detected in 47% of the broiler samples indicating presence of VRE at low levels, even though the use of avoparcin has been banned since 1995 in Denmark.

Among *Enterococcus faecalis* isolates from broilers, a significant decrease in resistance was seen for tetracycline, erythromycin, streptomycin and salinomycin, while among isolates from pigs only a significant decrease in tetracycline resistance was observed. The reduced occurrence of tetracycline resistance among all enterococci isolates from pigs can be related to the reduced consumption of tetracyclines.

The same type (ST16) of high-level gentamicin resistant (HLGR) *E. faecalis* was detected in pigs, pork, healthy humans, and from patients with infective endocarditis, indicating a zoonotic link.

In indicator *E. coli* isolates from pigs and broilers, no significant changes in the levels of resistance were observed from 2009 to 2010; however, the level of resistance to tetracycline in bovine isolates increased significantly from 2% to 9%. In 2010, no fluoroquinolone resistance was found in *E. coli* from Danish pigs and cattle, probably reflecting the low consumption since 2002 when the use in production animals was restricted by law. In contrast, 8% of the *E. coli* isolates from broilers were resistant to fluoroquinolone, which corresponds to the relatively higher use of fluoroquinolones in the broiler production during the last ten years compared with other production animal species.

Resistance in *E. coli* isolates from imported broiler meat was significantly higher compared with isolates from Danish broiler meat for 13 of the 16 tested antimicrobial agents. In 2010, ceftiofur resistance was observed for the first time in an isolate from Danish broiler meat, obtained without selective enrichment (1%), although significantly lower than among *E. coli* from imported broiler meat (7%). The occurrence of fluoroquinolone resistance in imported broiler meat (41%) was tenfold higher than in Danish broiler meat (4%).

In *E. coli* from Danish pork, resistance to sulfonamide decreased significantly from 38% to 19%. In 2010, significantly lower resistance to tetracycline and sulfonamide was found in isolates from Danish pork compared with imported pork. The resistance to fluoroquinolones remained low (one isolate) in Danish pork; in imported pork, 4% of the *E. coli* isolates were resistant to fluoroquinolones.

The occurrence of resistance in *E. coli* from imported and Danish beef was low.

ESBL-producing bacteria are resistant to extended-spectrum cephalosporins, which are often used for treatment of infections. Consequently, even a low occurrence of these bacteria can potentially be a serious problem. Using selective enrichment methods, the occurrence of ESBL-producing *E. coli* in pig farms, cattle and broilers at slaughter and in meat at retail was investigated. In production animals, the highest occurrence of ESBL-producing *E. coli* was found in broilers at slaughter (27%) despite no usage of cephalosporins in the Danish broiler production for at least a decade. In meat, the highest occurrence of ESBL-producing *E. coli* was found in broiler meat of imported (50%) and Danish (8.6%) origin. For imported broiler meat, the occurrence was significantly higher than in 2009.

The presence of ESBL-genes differed depending on animal reservoir. CMY-2 and SHV-2 seemed to be more related to the broiler production, whereas CTX-M-8 was found only in cattle. Several of the ESBL-genes detected among *E. coli* obtained from animals and meat can also be detected in *E. coli* of human origin. Broilers and broiler meat seem to be an important reservoir for ESBL-producing *E. coli*, also in countries like Denmark with no consumption of cephalosporins in the broiler production.

Broilers and broiler meat seem to be an important source for ESBL-producing *E. coli,* also in countries like Denmark with no consumption of cephalosporins in the broiler production. Resistance to vancomycin and quinupristin/dalfopristin still prevails at low levels among *E. faecium* isolated from pigs even though usage of these growth promoters has been banned for more than ten years.

Resistance in human clinical bacteria

Data on antimicrobial resistance in bacteria from diagnostic submissions are gathered by voluntary reporting from the DANRES group which covers the Departments of Clinical Microbiology (DCM) in Denmark. The only exceptions are methicillin resistant *Staphylococcus aureus* and invasive *Streptococcus pneumoniae* that are notifiable. Data on these bacteria are obtained from the reference laboratories at SSI.

Among *E. coli* blood isolates, resistance to 3rd generation cephalosporins was 7% in 2010, the same level as reported in 2009, but above the 2009 level in the other Nordic countries. Resistance to gentamicin increased to 6% in 2010. In 2010, ciprofloxacin resistance was 14% (min. 7%, max. 22% at the individual DCM), the same level as in 2009. No *E. coli* isolates from blood were

carbapenem resistant. Over the last decade, resistance to cefuroxime, ciprofloxacin and gentamicin has increased significantly. Resistance to 3rd generation cephalosporins has only been reported since 2008; during this period the resistance has increased.

In *E. coli* urine isolates obtained from hospitals, resistance to 3. generation cephalosporins was 5% in 2010, the same level as in 2009. Small decreases (one percent) in the occurrence of resistance were observed for the following antimicrobial agents: ampicillin (41%), sulfonamide (35%), ciprofloxacin (12%) and cefuroxime (2. generation cephalosporin) (5%).

In *E. coli* urine isolates obtained from primary health care**,** resistance to 3rd generation cephalosporins was 3% in 2010, the same level as in 2009. Nalidixic acid resistance increased significantly from 14% in 2009 to 15% in 2010. From 2009 to 2010, small (1-2%) but significant decreases in resistance were observed for ampicillin (40%) and sulfonamide (37%).

In *Klebsiella pneumoniae* blood isolates, 3rd generation cephalosporin resistance was 9% (min. 4%, max. 24%), the same level as reported in 2009. The level was above the level reported to EARS-Net by the other Nordic countries and corresponded to the occurrence reported by several other European countries in 2009 [EARS-Net 2009]. In the Eastern part of Denmark (Zealand), the occurrence of 3. generation cephalosporin resistant *K. pneumoniae* (14%) was significantly higher than in the Western part (Funen and Jutland) (6%). Fluoroquinolone resistance (ciprofloxacin 11%, nalidixic acid 17%) and aminoglycoside (gentamicin) resistance (6%) were above the levels reported from the other Nordic countries and the same as reported to EARS-Net by other European countries. When comparing 2009 with 2010, significant decreases were observed for gentamicin, ciprofloxacin and cefuroxime resistance; this was mostly due to decreased occurrence of these resistances in *K. pneumoniae* isolates from Zealand. This could in part be explained by interventions at hospitals in the Copenhagen area (Textbox 8). In 2010, carbapenem (meropenem) resistance was absent in *K. pneumoniae* blood isolates.

In *K. pneumoniae* urine isolates**,** 3rd generation cephalosporin resistance was 12% in isolates obtained from hospitals, and 7% in isolates obtained from primary health care, the same levels as reported in 2009. 3rd generation cephalosporin resistance in *K. pneumoniae* isolated from urine was significantly higher in the Eastern part of Denmark (Zealand) compared with the Western part (Funen and Jutland). A significant decrease in fluoroquinolone resistance (in 2010; ciprofloxacin 14%, nalidixic acid 20%) was observed from 2009 to 2010 among *K. pneumoniae* urine isolates from hospitalized patients. In the Eastern part of Denmark (Zealand), the occurrence of ciprofloxacin resistant *K. pneumoniae* in urine isolates from both hospitals and primary health care was significantly higher than in the Western part (Funen and Jutland).

Carbapenem (meropenem) resistance was present in the *K. pneumoniae* urine isolates from both hospitals and primary health care. One of the carbapenem resistant isolates produced the new carbapenemase enzyme New Delhi metallo-β-lactamase 1 (NDM-1) and was resistant towards all tested antimicrobial agents except tigecycline and colistin. The occurrence of carbapenem resistance is not mandatory reportable and no calculation of the frequency of carbapenem resistance could be made for *K. pneumoniae*. Sulfonamide resistance increased significantly among urine isolates from both hospitals (29% in 2010) and primary health care (34% in 2010).

ESBL-producing *E. coli* and *K. pneumoniae* are not mandatory reportable in Denmark and were only reported to the DANMAP report from a few DCMs; it is therefore not possible to calculate the frequency of this resistance.

Antimicrobial resistance in *Pseudomonas aeruginosa i*solates obtained from blood was low for all the tested antimicrobial agents.

Resistance to penicillins and erythromycin in *Streptococcus pneumoniae* and in Group A, B, C and G streptococci remained low in 2010.

In 2010, resistance to ampicillin increased to 92% in *Enterococcus faecium* isolates from blood. Vancomycin resistance was 1.8% in the *E. faecium* and less than 1% in the *E. faecalis* blood isolates. During 2010, an outbreak of vancomycin resistant (*vanA*) *E. faecium* was detected at Aarhus University Hospital. This outbreak is still under investigation. Only one of the DCM tested all enterococci from bloodstream infections for High-level gentamicin resistance (HLGR). Here, 36% of the tested *E. faecalis* isolates were HLGR, as were 74% of the tested *E. faecium* isolates.

In 2010, 1,418 cases of *Staphylococcus aureus* bacteraemia were reported, corresponding to 24.6 cases per 100,000 citizens. The number of methicillin resistant *S. aureus* (MRSA) was 20 (1.4%). This is at the same level as in 2009 and still among the lowest recorded incidences in Europe. The frequency of resistance towards fucidic acid and norfloxacin increased while resistance towards other antimicrobials was at the same level as in

The number of new cases of MRSA increased in 2010 to 1,097 compared with 817 in 2009. The number of cases was the highest reported in more than 25 years. The increase was recorded both among cases acquired abroad (247 in 2010 vs. 156 in 2009) and cases acquired in Denmark (852 vs. 661). Among Danish cases the increase was most marked in the group categorised as health-care associated, but with a community onset (HACO, 169 vs. 81). Of these, 129 cases did not report any known MRSA exposure and the increase may thus be attributed to a better completion of report forms. The number of hospital-acquired cases was still low and at the same level as in 2009 (62 vs. 53 cases).

Among the community-acquired cases, a significant change was recorded. In 2010, considerably more cases reported known exposure to MRSA, both patients with MRSA infections and carriers. The number of MRSA belonging to clonal complex CC398, which is associated with pigs, increased from 40 in 2009 to 109 in 2010. In

15 of these cases, no known contact to pigs or people with contact to pigs was reported. This may be an adaptation of the clone to the human host and the possibility for a human-to-human spread. The majority of the 15 persons lived in areas with recorded CC398 cases in humans and/or pigs. There are still no signs of spread to urban areas or spread through the food chain.

In 2010, a new gene conferring resistance to methicillin was recognised (*mecAlga251*). Previous methods failed to detect this new variant. In 2010, a total of 21 persons were demonstrated positive with this type. Investigation of MRSA strains from previous years showed that these strains have been spreading in Denmark since 2004.

The prevalence of MRSA was investigated in pigs at the farms, cattle and broilers at slaughter and in meat samples. The prevalence in pigs (16%) was at the same level as found among pigs at slaughter in 2009. This is lower than observed in some other European countries. MRSA was not found among cattle or broilers. The MRSA from pigs were CC398, and CC398 was found in 109 human cases, the majority in persons with contact to pigs. In 15 cases no direct contact was reported, whereas the majority was found in persons living in rural areas with known occurrence of MRSA CC398 in pigs. There are still no sign of spread of CC398 to urban areas. Imported meat still has the highest occurrence of MRSA (19%) as compared to Danish meat. The relatively frequent occurrence of MRSA in meat combined with no/very few cases in urban areas makes it safe to conclude that there is very little if any risk for meat being a risk for contracting MRSA CC398. Pigs still seem to be the most important reservoir for MRSA CC398.

Regarding blood and urinary tract infections in humans caused by *E. coli* and *K. pneumoniae,* 3rd generation cephalosporin resistance was at the same level as in 2009. Third generation cephalosporin and ciprofloxacin resistance were significantly higher in the Eastern part (Zealand) compared with the Western part (Funen and Jutland) of Denmark. An intervention study at Bispebjerg Hospital has shown that it is possible to decrease the number of resistant *K. pneumoniae* isolates. Most of the *E. faecium* isolates were resistant to ampicillin. The occurrence of resistance in *P. aeruginosa* and Streptococci was low. The number of hospital-acquired MRSA cases was still low and at the same level as in 2009, whereas the number of community-acquired cases increased. An increase in the number of human CC398 cases was observed, CC398 being associated with contact to

3 GENERAL INFORMATION

3. General information

The distribution of the Danish population in which antimicrobial agents were used in 2010 is displayed in Figure 3.1 together with the five health care regions and the 15 Departments of Clinical Microbiology (DCMs).

The amount of meat available for consumption in Denmark during 2007–2010 is presented in Table 3.1. Cooled and frozen fresh meat is included as well as natural-marinated broiler meat. The amount of domestically produced meat available for consumption in Denmark is estimated as production minus export.

Table 3.2 shows the antimicrobial agents that are registered for treatment of bacterial infections in animals and humans. Growth promoters, which are no longer used for animals in Denmark, are shown in parentheses. Most of the antimicrobial agents used for growth promotion in Denmark had effects on Gram-positive bacteria. Since 1995, the indicator enterococci from animals and meat (and in some years from healthy humans) have been used as a measure of resistance to the growth promoters.

Vibeke Frøkjær Jensen and Ulrich Stab Jensen

Table 3.1. Danish and imported meat available for consumption (in 100 Tons)[a], Denmark

DANMAP 2010

Source	Origin	2007	2008	2009	2010
Pork	Danish	1745	2113	1868	1819
	Import	516	956	833	873
Beef	Danish	730	831	845	922
	Import	800	812	888	1026
Broiler meat[b]	Danish	626	478	513	532
	Import	304	325	303	427
Turkey meat	Import	84	83	70	87

a) Source: Statistics Denmark. The volumes of Danish meat are estimated as production minus export
b) Natural-marinated broiler meat included

Figure 3.1. The five health care regions and 15 Departments of Clinical Microbiology (DCM) of Denmark

DANMAP 2010

North Denmark Region

No. of inhabitants	579,628
No. of inhabitants/km^2	73
No. of inhabitants/GP	1647

Central Denmark Region

No. of inhabitants	1,253,998
No. of inhabitants/km^2	96
No. of inhabitants/GP	1498

The Capital Region of Denmark

No. of inhabitants	1,680,271
No. of inhabitants/km^2	656
No. of inhabitants/GP	1536

The Sealand Region

No. of inhabitants	820,564
No. of inhabitants/km^2	113
No. of inhabitants/GP	1584

Region of Southern Denmark

No. of inhabitants	1,200,277
No. of inhabitants/km^2	98
No. of inhabitants/GP	1482

Source: Statistics Denmark (www.dst.dk) and the Danish Medical Association (www.laeger.dk). GP=general practitioner

Table 3.2. Antimicrobial agents marketed for systemic and veterinary intramammary therapeutic use in animals and humans, Denmark 2010

DANMAP 2010

ATC / ATCvet codes [a]	Therapeutic group	Antimicrobial agents within the therapeutic groups	
		Animals	Humans
J01AA / QJ01AA,QJ51AA	Tetracyclines	Chlortetracycline, doxycycline, oxytetracycline	Doxycycline, lymecycline, oxytetracycline, tetracycline, tigecycline
J01BA / QJ01BA	Amphenicols	Florfenicol	
J01CA / QJ01CA	Penicillins with extended spectrum	Ampicillin, amoxicillin	Ampicillin, pivampicillin, amoxicillin, pivmecillinam, mecillinam
J01CE / QJ01CE	Beta-lactamase sensitive penicillins	Benzylpenicillin, phenoxymethylpenicillin, procaine penicillin, penethamate hydroiodide	Benzylpenicillin, phenoxymethylpenicillin
J01CF / QJ51CF	Beta-lactamase resistant penicillins	Cloxacillin, nafcillin	Dicloxacillin, flucloxacillin
J01CR / QJ01CR	Comb. of penicillins, incl. beta-lactamase inhibitors	Amoxicillin/clavulanate	Amoxicillin/clavulanate, piperacillin/tazobactam
J01DB / QJ01DB,QJ51DB	First-generation cephalosporins	Cefalexin, cefadroxil, cefapirin	Cefalexin
J01DC	Second-generation cephalosporins		Cefuroxime
J01DD / QJ01DD,QJ51DD	Third-generation cephalosporins	Cefoperazone, ceftiofur, cefovecin	Cefotaxime, ceftazidime, ceftriaxone
J01DE / QJ51DE	Fourth-generation cephalosporins	Cefquinome	
J01DF	Monobactams		Aztreonam
J01DH	Carbapenems		Meropenem, ertapenem, doripenem
J01EA	Trimethoprim and derivatives		Trimethoprim
J01EB / QJ01EQ	Short-acting sulfonamides	Sulfadimidine	Sulfamethizole
J01EE / QJ01EW	Comb.of sulfonamides and trimethoprim, incl. derivatives	Sulfadiazine/trimethoprim, sulfadoxine/trimethoprim	Sulfamethoxazole/trimethoprim
J01FA / QJ01FA	Macrolides	Spiramycin, tylosin, tilmicosin, tylvalosintartrat, tulathromycin, gamithromycin	Erythromycin, roxithromycin, clarithromycin, azithromycin
J01FF / QJ01FF	Lincosamides	Clindamycin, lincomycin	Clindamycin
J01FG / QJ01XX [b]	Streptogramins	(Virginiamycin)	
J01G / QJ01RA,QA07AA	Aminoglycosides	Streptomycin, dihydrostreptomycin, gentamicin, neomycin, apramycin	Tobramycin, gentamicin
J01MA / QJ01MA	Fluoroquinolones	Enrofloxacin, marbofloxacin, difloxacin, ibafloxacin	Ofloxacin, ciprofloxacin, moxifloxacin
QJ01MB	Other quinolones	Oxolinic acid	
QJ01MQ [b]	Quinoxalines	(Carbadox, olaquindox)	
J01XA,A07AA / Not in ATCvet [b, c]	Glycopeptides	(Avoparcin)	Vancomycin, teicoplanin
J01XB / QA07AA [b]	Polypeptides (incl. polymyxins)	Colistin, (bacitracin)	Colistin
J01XC	Steroid antibacterials		Fusidic acid
J01XD,P01AB [c]	Imidazole derivatives		Metronidazole
J01XE	Nitrofurane derivatives		Nitrofurantoin
J01XX / QJ01FF	Other antibacterials	Spectinomycin	Methenamine, linezolid, daptomycin
QJ01XQ	Pleuromutilins	Tiamulin, valnemulin	
QP51AG04	Antiprotozoals, sulfonamides	Sulfaclozine	
Not in ATCvet [b]	Oligosaccharides	(Avilamycin)	
Not in ATCvet [b]	Flavofosfolipols	(Flavomycin)	

a) ATCvet codes starts with a Q

b) Animal growth promoters used before 1999 are listed in parentheses

c) Although intestinal antiinfectives (A07AA) and imidazole derivatives for protozoal diseases (P01AB) are used to treat human patients, they are not reported by DANMAP

ANTIMICROBIAL CONSUMPTION IN ANIMALS 4

4. Antimicrobial consumption in animals

4.1 Introduction

4.1.1 Demographic data

In 2010, the production of meat and dairy increased compared to 2009 (Table 4.1). The number of pigs produced (slaughtered or exported) increased by 3.3%, while the production in kg pork produced increased by 4% (Table 4.1), suggesting a slight increase in average weight at slaughter. This may be related to a decrease in number of sows in the last quarter. The export of fattening pigs (15–50 kg) has increased over the past years and at export, these pigs have received a large amount of antimicrobial agents relative to their bodyweight. Since 2006, more than 99% of the turkeys produced were exported for slaughter.

4.1.2 Policies and regulations of the use of antimicrobial agents in animals

Since the early 1990'ies there has been political and public focus on the use of antimicrobial agents in the Danish animal production. This led to the ban on avoparcin for growth promotion in 1994 and voluntary phasing out of the remaining antimicrobial agents for growth promotion during 1996–1999. In 2002, restricted use of fluoroquinolones was enforced. In July 2010, the pig industry imposed a voluntary ban on use of cephalosporins.

Regarding prescription medicines, a number of interventions affect the present antimicrobial consumption pattern in Denmark. Some of this legislation has had an evident influence on the prescription pattern, such as a steep decrease in consumption from 1994 to 1995 and a steep decrease in use of fluoroquinolones from 2001 to 2003. The effect of other parts of the legislation may be less obvious, but are important to keep in mind when interpreting the veterinary prescription patterns.

Table 4.1. Production of food animals and the production of meat and milk, Denmark

DANMAP 2010

Year	Broilers		Turkeys		Cattle (slaughtered)		Dairy cows		Pigs			Farmed fish[a]	
										Export		Fresh water	Salt water
	1000 heads	mill. kg	1000 heads	mill. kg	1000 heads	mill. kg	1000 heads	mill. Kg milk	1000 heads[b]	1000 heads[c]	mill. kg	mill. kg	mill. kg
1990	94560	116	571	2.5	789	219	753	4542	16425	-	1260	-	-
1992	107188	137	761	5.4	862	236	712	4405	18442	-	1442	35	7
1994	116036	152	1091	8.6	813	210	700	4442	20651	-	1604	35	7
1996	107895	149	961	9.3	789	198	701	4494	20424	-	1592	32	8
1998	126063	168	1124	11.6	732	179	669	4468	22738	-	1770	32	7
2000	133987	181	1042	10.3	691	171	636	4520	22414	-	1748	32	7
2001	136603	192	1086	13.2	653	169	623	4418	23199	-	1836	31	8
2002	136350	190	1073	12.8	668	169	611	4455	24203	-	1892	32	8
2003	129861	181	777	11.2	625	161	596	4540	24434	-	1898	34	8
2004	130674	181	1086	19.6	632	165	569	4434	25141	1712	1967	34	9
2005	122179	180	1237	17.4	549	145	559	4449	25758	2720	1988	31	8
2006	106182	163	785	11.3	509	140	556	4492	25763	3204	1957	29	8
2007	107952	178	1009	14.4	512	141	545	4515	26311	3522	2046	31	10
2008	107595	186	1068	12.3	509	138	559	4585	27078	4943	1985	30	10
2009	108851	181	1175	11.1	507	137	569	4734	27603	6642	1898	29	11
2010	117653	187	1184	14.0	519	142	574	4830	28505	7074	1974	-	-
Increase (%)[d]	8	3	1	26	2	3	1	2	3	7	4.0	-	-

Source: Statistics Denmark (www.dst.dk) and The Danish Directorate for Fisheries. All data include export of live animals for slaughter. Export data for poultry from Statistics Denmark (personal communication) and export of 15-50 kg pigs from Danish Agriculture and Food

a) The production of farmed fish includes fish transferred from one production facility to another. In 2009, this included 2.7 tonnes transferred between freshwater facilities (9.4% of the freshwater production), and 1.9 tonnes transferred from freshwater to salt water facilities, corresponding to 18% of the saltwater production

b) Including export of all age groups (not only for slaughter)

c) Export of 15-50 kg pigs. These are included in total number of heads, but antimicrobial use after export until slaughter is not registered as it takes place outside Denmark

d) Increase from 2009 to 2010

Official guidelines for choice of antimicrobial agents in pigs and cattle have been available for veterinarians since 1996. The guidelines provide specific recommendations for the selection of the appropriate antimicrobial agents for the treatment of all common indications in major production animal species. Initially, guidelines were developed by the National Veterinary Serum Laboratory (presently, the National Veterinary Institute). Since 2005, the guidelines have been updated by the Danish Veterinary and Food Administration (DVFA) in collaboration with the National Veterinary Institute, the National Food Institute, the Practicing Veterinarians Organization, University experts, and the Danish Agriculture and Food Council. The latest update is from 2010, see Textbox 1.

Relevant legislation regarding veterinary use of antimicrobial agents since 1993

Order (DK) 142/1993: Restricted the use of extemporaneously prepared medicines; also called the cascade rule, imposing mandatory first priority to medicinal products approved for the relevant species, subsidiary approved for other species.

Directive (DK) 60/1995: Limits the veterinarians' profit when distributing medicines to a maximum of 5-10% at sales.

Order (DK) 303/1995 and 304/1995: Limit the veterinary prescription to a maximum of 5 days of treatment in production animals. Exceptions only granted when a veterinary advisory service contract between the veterinarian and the farmer was signed. In those cases, up to 35 days of treatment is allowed for a diagnosed disease or a disease that was expected in the pigs, calves and poultry, on the basis of the veterinarian's knowledge of the herd (revised by Order (DK) 785/2010). By July 2010, veterinary advisory service contracts became obligatory for all larger pig and cattle herds in Denmark.

Order (DK) 303/1995: Treatment allowed only in diseased animals or animals in a well defined incubation period (metaphylaxis) and prophylactic use became illegal (revised by Order (DK) 910/2006).
Mandatory registration by the veterinary practitioners of used, delivered and prescribed drugs to farmed animals. The information must be available for inspection by veterinary officials for 3 years). Since 2001, reporting into the VetStat database has been mandatory.

Order (DK) 285/1996: Pharmacies and the pharmaceutical industry prohibited from offering economic incentives to veterinarians or others for the purpose of increasing product sales.

Order (DK) 119/2002: Flouroquinolones intended for injection were restricted to use by the veterinary practitioner only.

Order (DK) 134/2003: Mandatory susceptibility testing in relation to use of fluoroquinolones for production animals, documenting the need. Notification of use of fluoroquinolones to the authorities is mandatory.

Order (DK) 785/2010: Legal regulation of use of antimicrobial agents for mastitis in cattle (recommending using simple penicillins). A similar rule has applied since 2006, for part of the cattle herds, with "new health consultancy contract" [Order (DK) 1045/2006].

Order (DK) 1319/2010: The "yellow card" control of antimicrobial use in the pig production, imposing preventive measures in the herds with highest consumption per pig. In July 2010, an information letter about the upcoming "yellow card" was sent to the pig farmers, using the 20% highest number of ADD's per pig (Textbox 2).

One health evidence based prudent use guidelines for antimicrobial treatment of pigs in Denmark

Introduction: New prudent use guidelines for treatment of pigs in Denmark were presented at the Annual Meeting for the Danish Veterinary Hyologic Society in 2010. In May 2011, these new guidelines were published on-line.

The guidelines are based on available scientific evidence. For every combination of class of antimicrobial agents, swine disease and pathogen, an assessment of prudent use will be provided by the Danish Veterinary and Food Administration (DVFA) in a simple spreadsheet on-line. The spreadsheet presents all currently registered veterinary antimicrobial products for the specific diseases in drop-down lists with recommended dosages and treatment periods registered along with a colour coding indicating the most prudent choices based on efficiency, susceptibility, pharmacokinetics and human importance. The guidelines are dynamic lists, which can be changed on request or if new information or new antimicrobial agents emerge. The guidelines in Danish are found on the DVFA homepage (http://www.foedevarestyrelsen.dk).

Background: The new guidelines are the third step elaboration of prudent use treatment guidelines; the guidelines are part of the ongoing risk management strategy in Denmark for optimisation of antimicrobial consumption and reduction of antimicrobial resistance. The DVFA commenced elaboration of dynamic prudent use treatment guidelines for food-producing animals, starting with swine in 2005, followed by a reviewed concept for treatment guidelines for cattle in 2008 and now these new dynamic evidence based prudent use treatment guidelines.

The guidelines demonstrate the need for collaboration between the human and veterinary side in a one health concept in order to combat risk of development of resistant bacteria. The one health concept results from a strong collaboration between all stakeholders in a task force hosted by the DVFA. Members of the task force are: the Danish Veterinary Association, the Danish Animal Health Industry, epidemiologists and risk assessors from the Danish Meat Association and DVFA, researchers in pharmacology and swine diseases from the Faculty of Life Sciences, Copenhagen University as well as researchers from the Statens Serum Institut, the National Food Institute and the National Veterinary Institute.

Guidelines: Based on the available evidence, the different classes of antimicrobial agents are assessed by the following four criteria: a) clinical documentation of efficacy, b) susceptibility based on national microbiological data, c) pharmacokinetics and d) risk profiling of the human health concerns when the antimicrobial agents are used for veterinary antimicrobial treatment. In the new dynamic guidelines, the actual ranking is based on the scoring shown in Table 1, and the documentation for the ranking is included in the guideline spreadsheet.

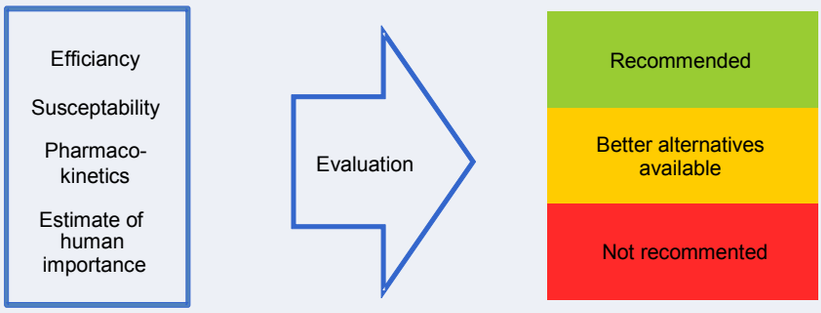
Table 1. Categorisation of antimicrobial agents in the new guidelines, Denmark DANMAP 2010

Category	Scoring
Efficiency	1 = Documented in summary of product characteristics (SPC) 2 = Documented in peer-revieved papers, EMEA or FDA papers
Susceptibility	Percent susceptible, among the isolates sent to the National Food Institute and National Veterinary Institute
Pharmacokinetics	Score based on MICKill/MIC50: 1 = range 0 - 0.19, 2 = range 0.2 - 0.39, 3 = range 0.4 - 0.59, 4 = range 0.6 - 0.79 and 5 = > 8.8. Based on estimated MICkill where 80% of the dosage are covered by a concentration above MIC50 estimated from the national data
Human importance	1 = very high, 2 = high, 3 = mediocre, 4 = low and 5 = very low. Follows FDA and OIE guidelines

The risk profiling of human health concerns is done according to the principles of FDA guidance 152 'Evaluating the safety of antimicrobial new animal drugs with regard to their microbial effects on bacteria of human health concern', as well as the principles in OIE-guidelines. For every antimicrobial group, it is estimated whether the probability for selection of antimicrobial resistance, exposure of humans and human consequences are very high, high, medium, low or very low. Based on these estimates, a common estimate for the human health consequences of the use of this antimicrobial group for swine is estimated. The common estimate gives a qualitative ranking of the expected future human health consequences by antimicrobial usage for swine of the different antimicrobial groups.

A new feature is that recommendations of usage of antimicrobial classes for the specific diseases (site of action) and specific pathogens are indicated by three different colours, green, yellow and red, in order to simplify the veterinary practitioner's choice of a prudent antimicrobial agent (Figure 1).

Figure 1. Prudent use guidelines for antimicrobial treatment of swine, Denmark



Green indicates antimicrobial agents that are recommended to be used for that specific disease and pathogen combination. The green labelled antimicrobial agents will have susceptibility above 80%, good pharmacokinetics and a risk profiling of human health consequences assessed to have no or only low consequences and preferably also evidence based documentation of clinical efficacy. Examples of green labelled antimicrobial agents with good judgement in all four categories for specific diseases and pathogens are: benzylpenicillinprocain, tiamulin, valnemulin and colistin sulfas. Yellow indicates antimicrobial agents that can be used, but where better alternatives are available.

Red indicates antimicrobial agents not recommended due high human health consequence or a very low susceptibility. Examples of red labelled antimicrobial agents are enrofloxacin, marbofloxacin, cefquinom and other cephalosporins, but also other antimicrobial agents with a low score on susceptibility for a specific pathogen will be labelled with a red colour.

To be used by the veterinary practitioners: The guidelines are directed towards veterinary practitioners. In Denmark, all veterinary medicinal products are prescription only and this places the veterinary practitioners as key persons in prudent antimicrobial usage. The veterinary practitioners may use the guidelines as a working tool in their counselling of preventive veterinary strategies in herds, thereby optimizing antimicrobial usage with due consideration to both human and animal health. They can look up a specific disease and pathogen in the guidelines; use the colours to choose a prudent antimicrobial and the drop-down lists to find products, dosage and treatment period. If they want to know how or why a certain treatment has a good or pour judgement in one or more of the categories, they can use the documentation spreadsheets included the guidelines to study the evidence behind.

For further information: Annette Cleveland Nielsen (acln@fvst.dk)

The yellow card initiative - special provisions for reduction of the antimicrobial consumption in pig holdings

Background: In order to reverse an increasing trend in antimicrobial consumption in the pig production and the related potential risk to human and animal health, the Danish Veterinary and Food Administration (DVFA) established the yellow card initiative in 2010.

As more than 80% of the antimicrobial agents used in livestock production in Denmark are used in the pig sector, the yellow card initiative was designed to target pig holdings with a high consumption of antimicrobial agents, beginning with the 5–10% of the pig producers with the highest antimicrobial consumption. In October 2010, the goal of a 10% reduction in consumption (in kg) in 2013 compared to the 2009 levels was set. The yellow card initiative is therefore an incentive for the pig producers to achieve this goal.

The yellow card initiative: Each year, the DVFA will issue threshold limits for antimicrobial consumption in three age groups of pigs. The limits for 2010 were as follows:

1. Weaners (7–30 kg): 28 Animal Daily Doses (ADD) per 100 weaners per day
2. Young pigs, including young females (over 30 kg), excluding sows, gilts and boars: 8 ADD per 100 pigs per day
3. Sows, gilts and boars: 5.2 ADD per 100 pigs per day

If the average antimicrobial consumption in a holding within a nine-month period exceeds one or more of the above threshold limits, DVFA may issue an order or injunction (the yellow card) compelling the owner of the holding, in collaboration with the veterinary practitioner, to reduce the antimicrobial consumption in the holding below the threshold limits within nine months.
In Denmark, prescription-only medicines can only be used and stored for a limited period of time. To prolong this period, the veterinarian must re-prescribe the medicine for a new limited period of time. However, during the nine-month injunction period, the DVFA may prohibit re-prescription of specific antimicrobial agents administered orally. In addition, the DVFA may also carry out one or more unannounced inspection visits to the holding during the nine-month period while the injunction is in effect.

If the antimicrobial consumption in the holding has not been reduced below the threshold limits after the expiry of the nine-month period, the DVFA may issue a second injunction compelling the owner of the holding to consult another veterinarian for second opinion advice on how to reduce the antimicrobial consumption below the threshold limits. This injunction may also be issued if the antimicrobial consumption in the holding has been reduced below the threshold limits within the nine-month period, but exceed the threshold limits during the succeeding 12-month follow-up period. The expert advice must include an action plan presenting specific interventions to reduce the antimicrobial consumption in the holding. As with the yellow card injunction, the DVFA may also prohibit re-prescription of specific antimicrobial agents administered orally and carry out unannounced inspection visits to the holding to ensure that animal welfare is not compromised by reduction in treatment of disease.

If the antimicrobial consumption in the holding has not been reduced below the threshold limits after the expiry of the second injunction period, the DVFA may carry out unannounced inspection visits to the holding within short intervals until the antimicrobial consumption in the holding has been reduced below the threshold limits.

The owner of a holding is required to pay a fee for each injunction or prohibition issued and for all inspection visits carried out in accordance with the special provisions. All other expenses, including the costs of veterinarian expert advice must also be paid by the owner.

Legislation: The requirements of the yellow card initiative are set out in Government Order No. 1319 of December 1st 2010 on special provisions for the reduction of the consumption of antibiotics in pig holdings.

For further information: Tim Petersen (tipe@fvst.dk)

4.1.3 Data sources

Data on antimicrobial use has been collected in Denmark since 1996, including historical data back to 1990. Until 2001, data were available on product level, based on report from the pharmaceutical industry of the total annual sales. In addition, sales of antimicrobial growth promoters and coccidiostatic agents approved as feed additives were reported by the feed mills to the Plant Directorate before 2001. Since 2001, consumption data presented in the DANMAP report have been obtained from the national monitoring programme, VetStat. Prior to 2001, data were based on overall sales figures from the pharmaceutical industry (Table AP1.0). In Denmark, all therapeutic drugs are prescription-only and VetStat collects data on all medicines prescribed by veterinarians for use in animals. Data on consumption of antimicrobial feed additives including coccidiostatic agents (non-prescription) and antimicrobial growth promoters (no longer in use) are also collected by VetStat. The VetStat database contains detailed data on all prescription medicines for animals, including item ID (Nordic Item Number), date of sale, recipient (farm-ID or practice ID), prescribing veterinarian (ID), species and age group, disease group a.o. (see Appendix 2 for further description).

4.1.4 Methods

The measures of antimicrobial consumption are numerous. For the description of trends within species, the number of Defined Animal Daily doses (ADD_{kg}) is preferred, because it describes the exposure independent of the choice of drug (see description in DANMAP 2009). ADD_{kg} is defined as the assumed average maintenance dose per day for treatment of one kg animal for the main indication in a specified species; correspondingly, the ADD_x, is the species specific assumed average maintenance dose for a standard body weight of x kg. The standard body weight is the assumed average body weight at treatment; e.g. for weaning pigs (7–30 kg) it is 15 kg, and the consumption for weaning pigs can be measured in ADD_{15} (see further detail in DANMAP 2009). However, doses are species specific, and have not been defined for all species; therefore, grams of active compound are used for measurement of overall consumption in DANMAP.

In this report, the production is used as the preferred denominator and is measured either in kg-meat-produced (including export of live animals) or in number of animals produced, i.e. slaughtered or exported. Population at risk (animal-year-at-risk) is used when looking specifically at consumption in sows or in dairy cows.

The number of pigs exported around 30 kg increased further by 7%, involving 25% of pigs produced in 2010. Because this production type has expanded importantly within a few years, it importantly affects the statistics. Particular for the evaluation of trends over time, data should be adjusted for such changes in production structure, as discussed in DANMAP 2009.

Pigs produced only to 30 kg contribute relatively little to the total production (by weight), while the amount of antimicrobials used for this part of the production (sows, piglets and weaning pigs) comprise two thirds of the total antimicrobial consumption for pigs. Thus, as illustrated in Figure 4.2, the consumption would be underestimated if merely divided by number of pigs produced. Conversely, the consumptions would be overestimated if the pigs exported at 30 kg were included in the production (by weight) (see also DANMAP 2009). The adjustment is based on the assumption that pigs exported at 30 kg compared to those not exported, on average received the same amount of antimicrobial agents before export, as other pigs from farrowing to 30 kg, giving a more robust measure of production (see Section 2.1 in Appendix 2)

4.2 Total antimicrobial consumption

Table 4.2 shows the total antimicrobial consumption in production animals from 2001 to 2010, with a decrease from 128.3 tonnes in 2009 to 125.5 tonnes in 2010. Due to the phasing out of antimicrobial growth promoters and legal regulations of the therapeutic use implemented in the 1990'ies, the overall antimicrobial consumption in production animals decreased importantly in that decade (Appendix 1, Figure AP1.1 and table AP1.0). During the period 2001–2009, the consumption in production animals increased gradually by 36%, but in 2010, the consumption was still 40% lower than in 1994, before the aforementioned interventions had been implemented (Table 4.2). In addition, the meat production has increased by 17% since 1994 (Table 4.1).

In 2010, the total veterinary consumption of antimicrobial agents including companion animals amounted to 126.9 tonnes (see details in Table 4.3), representing a 2.1% decrease compared to 2009. The decrease was mainly attributable to a decrease in consumption in pigs despite the increase in production (Table 4.1). The distribution of total consumption of antimicrobial agents among the major animal species has not changed importantly from previous years (Figure 4.1). In 2010, the antimicrobial consumption in pigs, cattle and poultry comprised 79% (100.5 tonnes), 12% (14.6 tonnes), and 0.7% of the total veterinary consumption, respectively.

For pigs, the adjusted figures are used to describe temporal trends, while non-adjusted figures are used to describe the relative consumption of different antimicrobial agents within individual years.

Table 4.2. Estimated total consumption (kg)[a] of prescribed antimicrobial agents for production animals[b], Denmark

DANMAP 2010

ATC$_{vet}$ group[c]	Therapeutic group	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
QJ01AA	Tetracyclines	28500	24500	27150	29350	29550	31800	36600	35400	38400	35550
QJ01CE	Penicillins, b-lactamase sensitive	16400	17500	18950	20900	22250	22650	23850	23950	25950	27100
QJ01C	Other penicillins	8600	9500	10600	12300	11650	10950	10900	10550	12000	12450
QJ01D	cephalosporins	100	150	200	250	250	250	300	300	250	200
QJ01EW	Sulfonamides + trimethoprim	9550	10550	10600	11500	12200	13700	13800	13300	14950	13900
QJ01EQ	Sulfonamides	950	900	850	850	750	750	700	600	4505	550
QJ01F	Macrolides, lincosamides	13400	13650	14000	16150	15300	14350	16500	15250	17350	16800
QJ01XQ	Pleuromutilins	4050	4500	5400	6600	6500	6350	6100	9200	10650	10700
QJ01G/ QA07AA	Aminoglycosides	11600	11700	11750	11650	10800	10600	8100	6000	6300	6200
	Others	900	1600	1400	950	1200	1200	1150	1650	1900	2100
Total		94000	94700	100900	110500	110400	112700	118000	116100	128200	125500

Data source VetStat. Only veterinary drugs are included (including parenteral treatment in companion animals). Veterinary drugs almost exclusively used in pets (tablets, capsules, ointment, eye/ear drops) are excluded. Dermal spray with tetracycline, extensively used in production animals, is the only topical drug included

a) Kg active compound rounded to nearest 50 for antimicrobial classes and 100 for totals

b) Included also the parenteral use in companion animals

c) Only the major contributing ATCvet groups are mentioned

Table 4.3. Antimicrobial agents sold (kg active compound)[a] by animal species and age group, Denmark

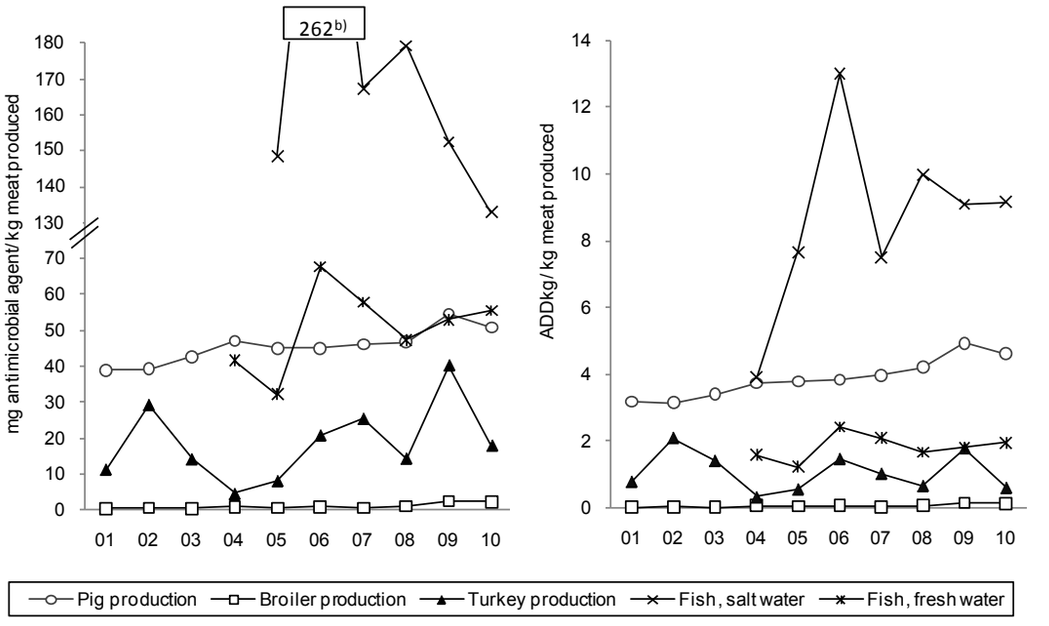
DANMAP 2010

Therapeutic group[b]	Amcol	Amglc	Ceph	FQ	Quinol	Linco	Macro	Pleuro	Pen-b-sens	Pen-other	Sulfa-TMP	Tet	Others	Total
ATCvet groups[c]	QJ01 B	QJ01 G	QJ01 DA	QJ01 MA	QJ01 MB	QJ01 FF	QJ01 FA	QJ01 XX	QJ01 CE	QJ01 CA	QJ01 E	QJ01 AA	QJ01 X	
Pigs, total	*150*	*4950*	*49*	*0*	*0*	*2663*	*12978*	*10661*	*18323*	*9011*	*8914*	*32318*	*510*	*100527*
- Sows and piglets	118	2191	41	0	0	692	888	1477	9764	4627	7278	3004	70	30150
- Weaners	23	2449	5	0	0	829	7398	3928	1679	2904	1487	17464	431	38597
- Finishers	9	294	2	0	0	1136	4651	5232	6849	1450	138	11785	7	31552
- Age not given	0	16	0	0	0	6	42	25	31	29	11	65	1	228
Cattle, total [d]	*390*	*832*	*117*	*1*	*0*	*28*	*216*	*16*	*7754*	*1361*	*2063*	*1845*	*11*	*14636*
- Intramammaries	0	30	69	0	0	3	0	0	189	157	8	0	2	457
- Cows and bulls	18	374	42	0	0	2	143	0.1	6997	803	1112	1280	1	10773
- Calves<12 months	362	328	2	0.1	0	6	66	0.2	364	197	474	497	7	2304
- Heifers, Steers	9	21	1	0	0	0.4	5	0	173	26	47	62	0	344
- Age group unknown [e]	1	79	3	1	0	16	2	16	32	179	422	5	1	757
Poultry, total	*6*	*4*	*0*	*0*	*0*	*2*	*202*	*12*	*31*	*270*	*109*	*242*	*1*	*879*
- Broilers	0	0	0	0	0	0	39	0	19	189	8	82	0	338
- Breeding and rearing, broilers	0	0	0	0	0	0	2	0	12	39	3	34	0	91
- Layers incl. rearing	0	0	0	0	0	0	13	11	0	21	0	2	1	48
- Turkeys	5	0	0	0	0	0	144	0	0	4	5	94	0	252
- Geese and ducks	0	0	0	0	0	0	0	0	0	0	0.2	14	0	14
- Gamebirds	0.4	4		0.1		2	3	1	0	15	87	11	0.1	124
- Species unknown	0.1	0	0	0.1	0	0	1	0.1	0.1	1	5	5	0	12
Other production animal species														
- Small ruminants	0	2	0.1	0	0	0	0.1	1	4	5	2	17	0	31
- Fur animals	0.1	284	0.2	0.4	0	135	579	0	2	1533	382	798	0.4	3714
- Aquaculture	190	0.1	0	0	838	0	0	0	0	5	2026	1	0	3060
- Other production animals	0	3	0.2	0.1	0	1	0	0	3	1	14	3	0	25
species unknown[f]	0	0	0	0	0	0.2	-2	0.5	1	3	0.4	1	0.1	3
Companion animals, total	*8*	*74*	*321*	*14*	*0*	*63*	*30*	*4*	*645*	*674*	*1047*	*167*	*37*	*3084*
- Pet animals	7	55	317	13	0	62	22	2	469	667	456	144	37	2252
- Horses or pets	1	18	3	1	0	0.3	8	2	163	7	484	21	0	708
- Horses	0.1	1	0.4	0	0	1	1	0	12	0.3	106	2	0.1	124
Species unknown[g]														
- Systemic use	0	55	2	3	0	0	-25	8	382	170	133	163	1	892
- Topical drugs	0	4	0	0	0	0	0	0	0	0	0.2	39	6	49
- Intramammaries	0	1	2	0	0	0	0	0	4	7	1	0	0.1	15
Total	744	6209	491	19	838	2891	13978	10703	27149	13038	14691	35595	567	126915

a) Amounts over 0.5 kg are given with one decimal; amounts of 0.0 kg are given as 0
b) Amcol=amphenicols; Amglc=aminoglycosides; Ceph=cephalosporins; FQ=fluoroquinolones; Quinol=other quinolones; Linco=lincosamides; Macro=macrolides; Pleuro=Pleuromutilins; Pen-β-sens=beta-lactamase sensitive penicillins; Pen-other=penicillins with extended spectrum, cloxacillin and amoxicillin/clavulanic acid; Sulfa-TMP=sulfonamides+trimethoprim; Tet=tetracyclines. Sulfaclozin (a prescription coccidiostat) is included in the sulfonamide/trimethoprim group
c) Only the ATC group contributing mostly to the antimicrobial group is mentioned. Combination drugs are divided into active compounds
d) in 2010, half of the prescribed antimicrobials for cattle was purchased at pharmacies; half was either administered or handed out by veterinary practitioners. Reporting from large animal practice on medicines for cattle is validated against data on medicines sold from pharmacies to cattle practice (not mixed practice), and the proportion in accordance is included under the respective age groups. Medicines sold to cattle practice, but usage not reported by a veterinarian are included under "age group unknown"
e) About 10% of the pharmacy sales for use in practice is not reported (possibly due to factor errors at reporting), when used or sold in practice, amounting to 700 kg in 2010. In addition, part of pharmacy sales to farms are lacking age group ID, amounting to 55 kg in 2010
f) Sales to farmers (valid farm ID codes) given, but animal species not identifiable. Negative numbers are due to prescribed antimicrobials that were not picked up by the farm owner and where the records the species identity is missing
g) Negative numbers are due to over-reporting by veterinarians of antimicrobial medicines used in practice to specific species

Figure 4.1. Antimicrobial consumption per kg meat produced from pigs[a], broilers, turkey and aquaculture[b], Denmark

DANMAP 2010



a) Export of animals for rearing or slaughter is included. However, data for pig production is not adjusted for the increasing export of pigs at 30 kg body weight, although the body mass at export is included in the production (see text). Thus, the figures overestimates the increasing consumption in the pig production in particular during the last three years

b) In 2006, the consumption reached 262 mg/kg fish produced in salt water, related to unusually warm summer months. The doses for fish are defined as 30 mg/kg for sulfonmide-trimethoprim, 10 mg/kg for oxolinic acid and 15 mg for florphenicol (source: Danish Aquaculture)

4.3 Antimicrobial consumption by animal species

A comparison of trends in antimicrobial use, per species, is shown in Figure 4.1; the trend lines differ depending on the unit used. For example, for turkeys the peak in 2009 is highest using mg, whereas the peak in 2002 is higher using the ADD, due to shift from amoxacillin towards tetracyclines. Correspondingly, the consumption in aquaculture is extremely high compared to other species when measured in mg, but more similar to the consumption in pigs (at least in some years) when measured in ADDkg, because the dominant drug of choice in aquaculture is used in a very high dosage (30 mg/kg).Trends may also be affected by shifts in the production. In this report, number of pigs produced has been used as the denominator, adjusted for changes in production as described above. The effect of using this measure is shown in Figure 4.2.

For comparison between species, kg meat produced has been used; this measure overestimates the selection pressure in species with long lives (e.g. cattle) relative to species slaughtered at an early age (e.g. poultry). Alternatively, live biomass could be used for comparison of selection pressure between species. Because cattle in Denmark are almost entirely dairy cattle, living many years after reaching the slaughter weight, the consumption cannot be directly compared with other species using 'kg meat produced' as the denominator.

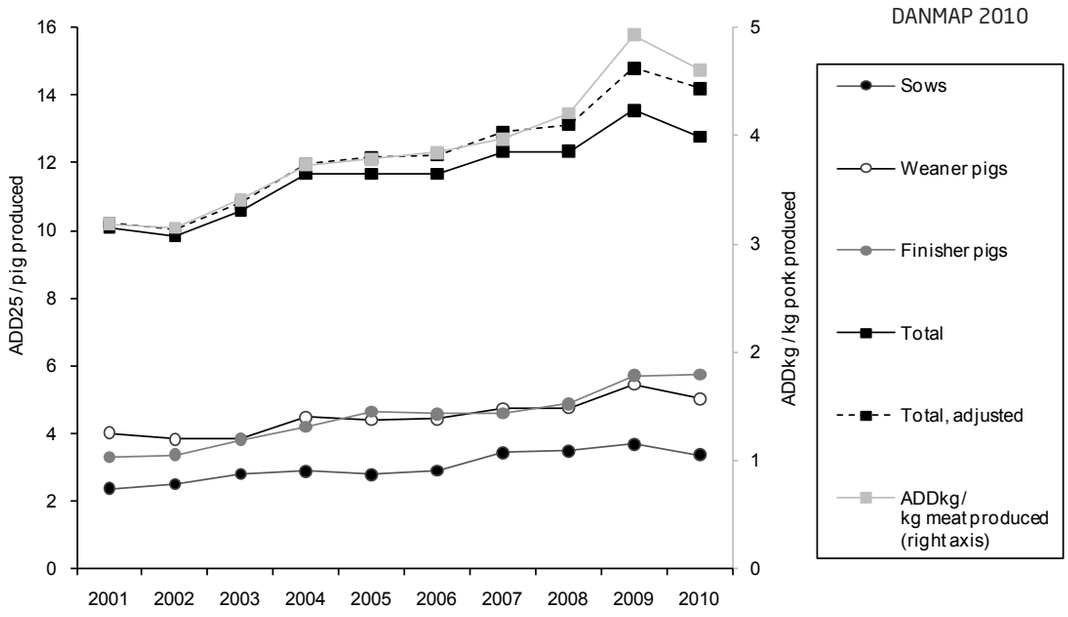
4.3.1 Antimicrobial consumption in pigs

In 2010, the total antimicrobial consumption in pigs was 100.5 tonnes active substance (Table 4.3), a decrease of 3 tonnes compared to 2009. Relative to the meat production, including live export, the consumption decreased to 50.9 mg/kg or 4.6 ADD_{kg}/kg pork produced (Figures 4.1 and 4.2). Consumption of antimicrobial agents for systemic use in pigs (2001–2010), given as Animal Daily Doses (ADDs) to the different age groups, are presented in Appendix 1 (Table AP1.1).

In 2010, the overall consumption for pigs decreased by 5% in ADD_{kg} per pig produced (adjusted for live export, Figure 4.2). The decrease was mainly related to a 5% decrease in consumption of tetracyclines. However, large relative reductions were also observed for macrolides (2%), aminoglycosides (16%), lincosamides/spectinomycin (7%) and cephalosporins (48%) (Figures 4.3 and 4.4). The 2010 overall decrease in consumption was entirely related to the second half of the year. During the first six months the consumption increased by 8% (in ADD_{kg}) compared to the same period in 2009.

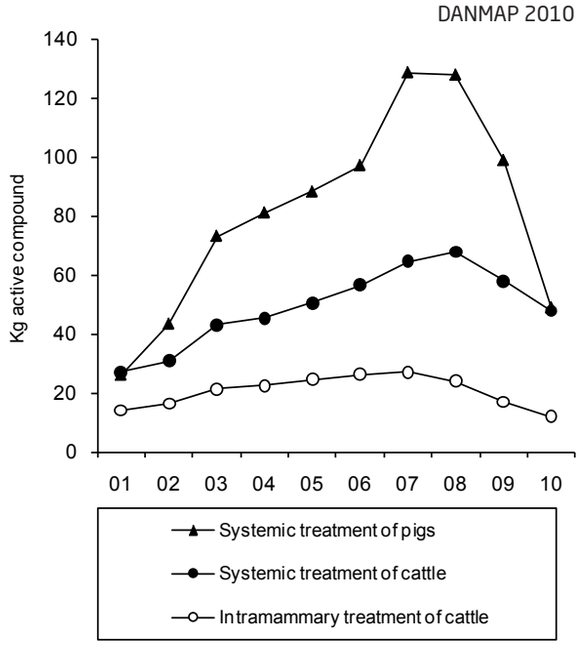
Regarding cephalosporins, the decrease was related to a voluntary ban by the industry, enforced in July 2010 (Figures 4.3 and 4.4). As in previous years, the cephalosporins were mainly (86%) prescribed for sow herds, including use in piglets for expected outbreaks of umbilical infection (DANMAP 2007). Also in July 2010, an information letter about the upcoming "yellow

Figure 4.2. Consumption of antimicrobial agents in sows, weaner pigs[a], finishers[b] and total pig production[c], given as Animal Daily Doses (ADDs), Denmark



a) Consumption in sows and weaners is given as ADD25 divided by number of weaning pigs produced
b) Consumption in finishers is given as ADD25 divided by number of finisher pigs produced
c) The" adjusted total" is given with the same units as the total, but adjusted for the increasing export of pigs at 30 kg (see text). Consumption per kg meat produced is given as ADDkg divided by meat production including live export

Figure 4.3. Consumption of 3rd and 4th generation cephalosporins in pigs and cattle, Denmark



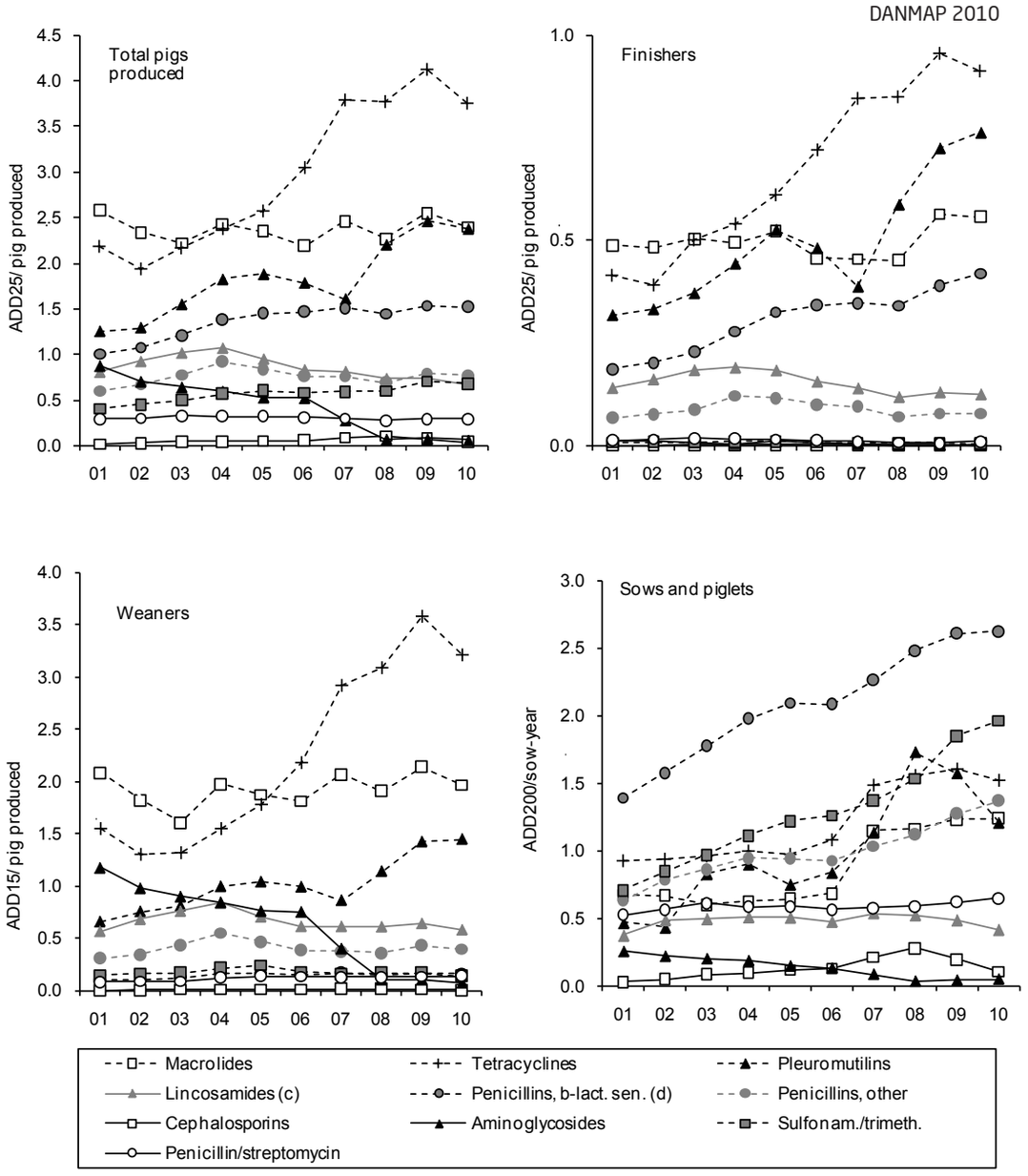
card" was sent to the pig farmers, using the 20% highest number of ADD's per pig (Textbox 2). This is a likely explanation for the overall reduction in consumption of 14% in July alone, and a 13% reduction in the second half year compared with the same period in 2009 (measured in ADDkg).

However, since 2001, the consumption has been gradually increasing, and during the past decade, the total consumption in pigs increased by 39% (adjusted for live export, Figure 4.2). Tetracyclines, macrolides and pleuromutilins, mainly for oral therapy, continued to be the most commonly used antimicrobial agents in pigs throughout 2001–2010(Figure 4.3).

From 2001–2009, the use of tetracyclines in ADD25 per pig produced increased by 100% (adjusted), followed by the 8% decrease in 2010; both trends have mainly been driven by trends in prescription for weaning pigs but also in finishers (Figure 4.4). Since 2005, tetracyclines have been the most common antimicrobial agent used in pigs, likely affected by treatment guidelines launched by the veterinary authorities that year, recommending that tetracyclines should be preferred over macrolides (critically important in human medicine), when a first priority agents could not be used.

Over the past decade, the consumption of macrolides has fluctuated between 2.2–2.6 ADD25 per pig produced, with no clear trends. The overall consumption of pleuromutilins was similar in 2009 and 2010, after a 53% increase since 2007 when prices for tiamulin were reduced making it price competitive with macrolides and tetracyclines. However, the consumption

Figure 4.4. Antimicrobial consumption[a] in the pig production[b], Denmark



Note: Amphenicols, colistin, fluoroquinolones, intramammaries, gynecologicals and topical drugs are not included in the figure. Data from veterinary practice are not included (<1% of the consumption in pigs)

a) ADD25: doses for treatment of 25 kg pigs, to compare treatment across age groups. ADD15: Doses for treatment of 15 kg pigs, the assumed average dose for treatment of weaners (7.5–30 kg). ADD50: Doses for treatment of 50 kg pigs, the assumed average dose for treatment of finishers (30–110 kg), estimate based on the number of pigs produced excluding those pigs exported at 15–50 kg. ADD200: Doses for treatment of 200 kg pigs: the medicines are used either in sows (bodyweight>200 kg) or in piglets (<2 kg–7.5 kg)

b) Total pigs produced includes pigs exported at 30 kg, which has increased in numbers from 1.7 million in 2004 to 7.1 million in 2010, comprising 25% of the total consumtion, although consumption in these pigs is included only from birth to 30 kg body weight. See discussion in the text

c) Beta-lactamase sensitive penicillins

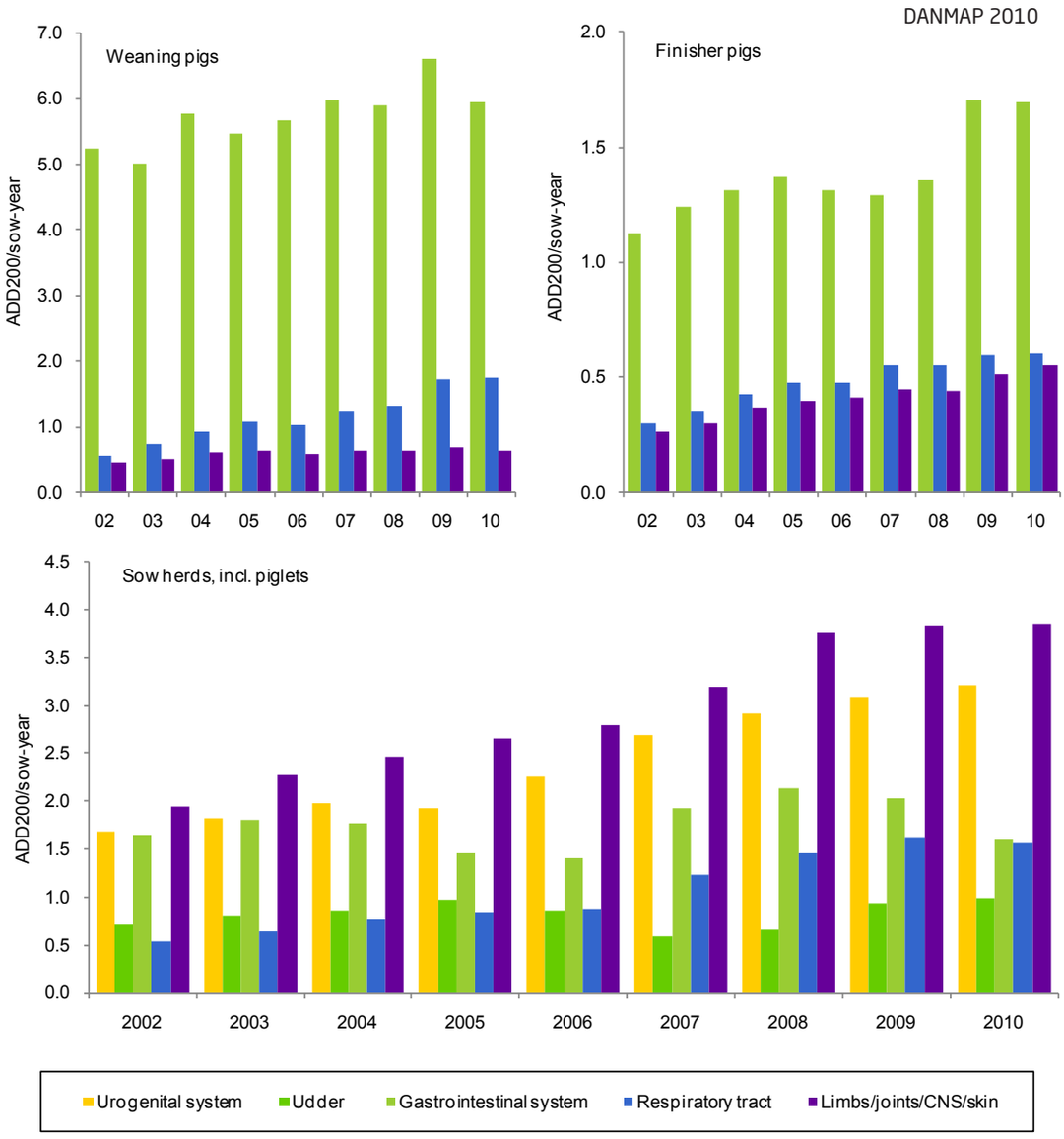
d) Lincosamide/spectinomycin combinations comprise 65% of this group

of pleuromutilins increased by 5% in finishers, while the consumption in sow herds decreased by 23%. Pleuromutilins have been recommended for sanitation against *Brachyspira hyodysenteriae* in sow herds in Denmark, and the decrease in use of pleuromutilins was related to the overall decrease in prescription for gastrointestinal disease in sow herds (Figure 4.4).

The overall decrease in consumption in pigs was mainly due to a decrease in consumption in weaning pigs but also in sows (Figure 4.4). In both age groups, the decrease was related to a decrease in prescriptions for gastrointestinal disease. In weaning pigs, the prescription for gastrointestinal infections is the major indication (70% in 2010), and decreased by 10% from 6.6 ADD15 in 2009 to 5.9 ADD15 per pig produced in 2010, reaching the level from 2008; the overall prescription for weaning pigs decreased from 9.1 ADD_{15} to 8.4 ADD15 per pig produced. In sow herds, the prescription for gastrointestinal disease decreased by 22% from 2.0 ADD200 to 1.6 ADD200/sow-year, while the total consumption decreased from 11.6 ADD200 to 11.3 ADD200 per sow-year. In finishers, the prescription was 2.9 ADD50 per finisher produced both in 2009 and 2010, and no important changed occurred in indication (Figure 4.5). However, a large increase in prescription for gastrointestinal disease was seen from 2008 to 2009. In all age groups, the prescription for respiratory disease has been increasing throughout the last decade (Figure 4.5).

It should be noted that these statistics are derived from the information on indication (disease group) in the VetStat database. The indication is entered by

Figure 4.5. Antimicrobial consumption by indication[a] for sows/piglets, weaner and finisher pigs, Denmark



a) The indication given on the prescription. At prescription, VetStat codes are used, representing the target organ system. In this figure, indications representing <0.1ADD (per sow year or pig produced) is not shown

Table 4.4. Consumption of antimicrobial agents for intramammary application in cattle, Denmark

DANMAP 2010

Antimicrobial agents	ATC_{vet} classes	2005	2006	2007	2008	2009	2010
		ADD (1000's)[a]					
Penicillins[b]	QJ51CE, QJ51CF, QJ51CR	454	464	454	506	599	671
Aminoglycoside-benzylpenicillin combinations[c]	QJ51RC	231	203	202	201	220	176
Cephalosporins, 1st generation	QJ51DA	206	197	177	169	178	174
Cephalosporins, 3rd and 4th generation	QJ51DC, QJ51DD	229	255	262	231	156	105
Others[d]		42	35	31	30	27	44
Total		1163	1153	1126	1138	1180	1170
ADD/cow-year		2.08	2.07	2.07	2.04	2.07	2.04

a) For intramammary treatment, 1 ADD is defined as the dose to treat one teat for 24 hours

b) Includes benzylpenicillin, cloxacillin, and cloxacillin-ampicillin combinations

c) Mainly dihydrostreptomycin-benzyl benicillin combinations; includes also combinations of penicillin/aminoglycoside with bacitracin or nafcillin

d) Lincosamides, neomycin-lincomycin combinations and trimethoprim-sulfonamide combinations

the veterinarian and refers to either the current or expected disease within a 30 day period (based on his/her knowledge of the herd). However, the prescribed antimicrobial agents may in some cases be used for other diseases than originally indicated on the prescription, in which case it is re-prescribed by the veterinarian at the farm. Such re-prescriptions are not registered in VetStat.

4.3.2 Antimicrobial consumption in cattle

In 2010, it was estimated that14.6 tonnes of antimicrobial substance were prescribed for cattle, as compared to 15 tonnes in 2009, representing a 3% decrease (Table 4.3). These estimates are based on pharmacy data, including prescription for cattle and cattle practice

Consumption of antimicrobial agents for systemic use in cattle (2005–2010), given as Animal Daily Doses (ADDs) to different age groups, are presented in Appendix 1 (Table AP1.2). For cattle, data are only shown for the period 2005–2010, because data quality was not acceptable before 2005 due to reporting errors. Before 2006, the majority of medicines for cows were purchased through veterinary practices, and in 2002–2006, up to 20% of some of the antimicrobial used in practice were missing due to technical errors in the amounts reported and the data transmissions. However, in 2010, underreporting from veterinary practice had reached a level of 5–10%, corresponding to an overall 2–5% underreporting on age group level. Thus, information about age groups was available for 95–98% of the total consumption.

Pharmacy data have a very high quality, and the overall improvement of data quality for cattle is also a result of an increasing proportion of the medicine being purchased through the pharmacies. In 2010, approximately half of the antimicrobial agents for cows were purchased through the pharmacies.

Based on the type of technical errors, we assume that the missing data are random, and thus that the data from the veterinarians are representative for the relative choice of drugs over time.

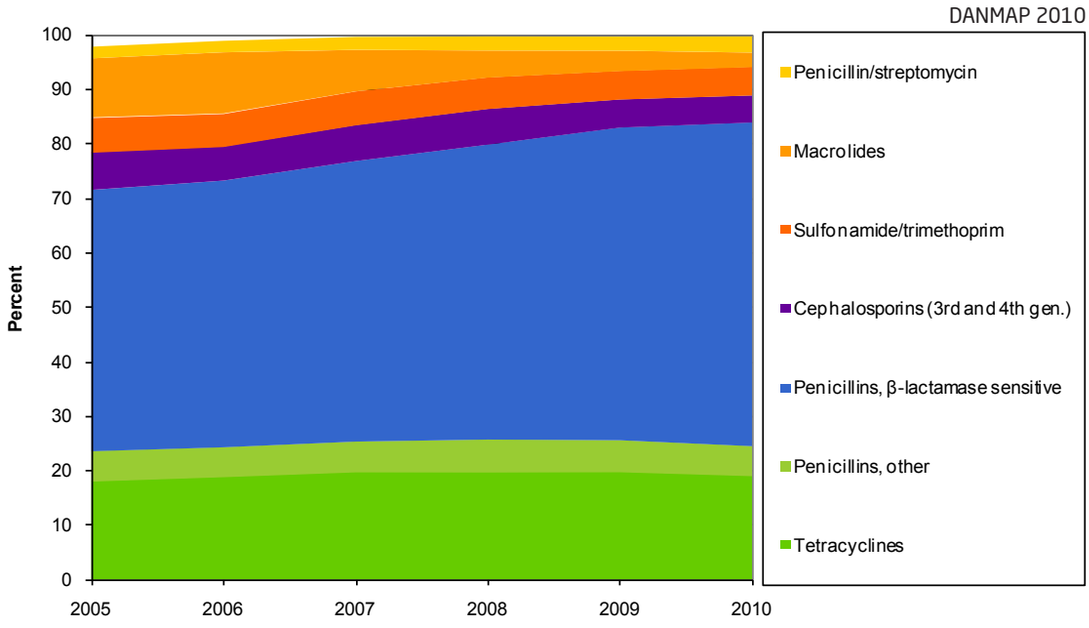
From the pharmacy sales, data for cattle practice, and thoroughly validated data from veterinary practices, it can be concluded that the antimicrobial consumption in cattle has been stable, between 14–15 tonnes during 2005–2009. During this period, the veal and beef production has decreased by 2% and the milk production has increased by 9% (Table 4.1).

The temporal trend in choice of drug for systemic use is shown in Figures 4.6 and 4.7. In 2010, the relative use of beta-lactam sensitive penicillins for systemic use in cows increased by 3.5%, mainly related to a relative decrease in the usage of tetracyclines (4%) and macrolides (29%). The major indication for treatment of cows was mastitis (64% of systemic use). Also for intramammary treatment, penicillins - mainly narrow spectrum (beta-lactamase sensitive and beta-lactmase resistant) - was the major class, comprising 57% of the treatments (Table 4.4). Combinations of benzylpenicillins (mainly with dihydrostreptomycin) comprised an additional 15%.

In cows and bulls, the proportional use of beta-lactamase sensitive penicilins has increased from 48% in 2005 to 59% of the overall consumption in 2010, while the use of macrolides decreased from 11% to 3% of the consumption for systemic use in cows. This is in accordance with the official guidelines.

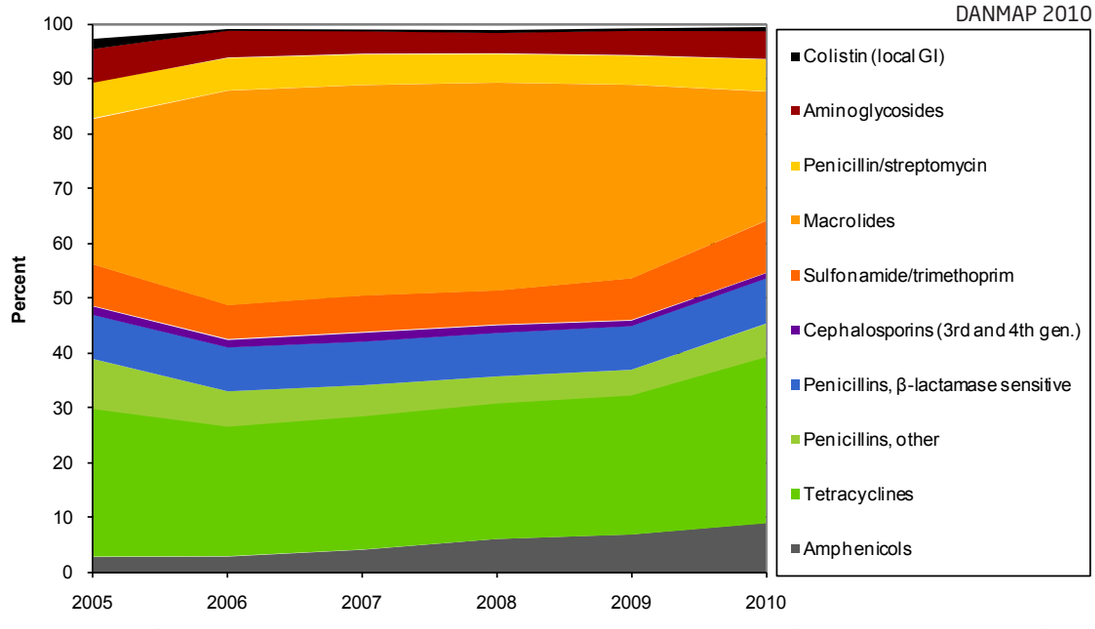
The use of intramammary treatment has been stable during 2005–2010, while the proportion of narrow spectrum penicillins increased from 39% to 57% of the treatments (Table 4.4). From 2005 to 2010, the use of intramammary treatments has decreased by 2% measured in ADD per cow-year.

Figure 4.6. Proportional consumption (in ADD) of antimicrobial agents[a] for systemic treatment in cows and bulls, Denmark



a) The antimicrobials not shown (amphenicols, fluoroquionolones, lincosamides, aminoglycosides, colistin and pleuromutilins), each account for less than one percent of the consumption

Figure 4.7. Proportional consumption (in ADD) of antimicrobial agents[a] for systemic treatment in calves, Denmark



a) The antimicrobials not shown (fluoroquionolones, lincosamides, and pleuromutilins), each account for less than one percent of the consumption

In calves, the major drug of choice in 2010 was tetracyclines (mainly oxytetracyclines), and the consumption increased from 26% in 2009 to 30% in 2010. From 2006 to 2009, macrolides were the most frequently used class, but the use was reduced from 35% in 2009 to 24% in 2010, in accordance with the official guidelines. The major indication in calves was respiratory disease (64% of systemic use).

The use of fluoroquinolones in cattle was only 1 kg active compound, and has remained at a low level since 2003. As in 2009, the consumption of 3rd and 4th generation cephalosporins decreased in 2010 both in cows and in calves. Systemic use decreased by 17% and intramammary use decreased by 29%. Overall since 2008, when the consumption measured in kg active substance was highest, the consumption of 3rd and 4th generation cephalosporins for systemic and intramammary use has decreased by 29% and 50% (Figure 4.4, Table 4.4). These trends in choice of antimicrobial agents for intramammary treatment may reflect a response to debate, guidelines and information in recent years, about the consequences regarding development of ESBL. However, they may also be the result of new regulations that require testing for antimicrobial resistance in cases where antimicrobial agents other than simple penicillins are prescribed for mastitis.

4.3.3 Antimicrobial consumption in poultry

In Denmark, the poultry production is comprised mainly of broiler production (*Gallus gallus*), followed by egg layers (*Gallus gallus*) and turkey production. In addition, there is a minor production of ducks, geese, and game birds, while pigeons are kept for sports. Consumption of antimicrobial agents for systemic use in poultry (2001–2010), given as Animal Daily Doses (ADDs) to the different species, are presented in Appendix 1 (Table AP1.3).

In 2010, the total antimicrobial consumption in poultry was 879 kg active substance (Table 4.3), representing an 18% decrease compared to 2009. However, this was still higher than the levels in previous years (2001–2008). In general, increasing disease problems caused a steep increase in antimicrobial consumption for poultry in 2009 (see DANMAP 2009); these disease problems seems to be under control in 2010, as indicated by the decrease in antimicrobial consumption, both for the layers, rearing for broiler production, and the turkey production; however, further increased use was observed in broilers.

The antimicrobial consumption in domestic fowl (*Gallus gallus*) is generally at a very low level (Figure 4.8). Therefore, few disease outbreaks in some farms importantly affect the national consumption in domestic fowl, causing considerable fluctuations in annual consumption.

The total consumption in broilers in 2010 was 429 kg including breeding and rearing (Table 4.3). For broilers, an additional increase in consumption was observed in 2010, mainly in the prescription of amoxicillin, which was still the major drug of choice (Figure 4.8). Prescription for 79 broiler farms was registered, corresponding to 28% of the broiler farms [Statistics Denmark 2011]. According to some of the major poultry practitioners, at least part of the increase in antimicrobial consumption was related to outbreaks of diarrhoea associated with coccidiosis, due to treatment failure (resistance to salinomycin). Due to the decrease in consumption in the parent and grandparent flocks (breeding and rearing), the overall consumption (0.14 ADDkg/kg meat produced) in the broiler production was at the same level as in 2009, but two–five times higher than in previous years (Figure 4.8).

The antimicrobial consumption in the layer production (*Gallus gallus*) is quite low, and the total consumption in 2010 was only 48 kg (Table 4.3). Measured in ADD_{kg} per kg eggs produced, the consumption in layers including rearing in 2010 was at the same level as in 2009, a level three to four times lower than in the broiler production, however the production (meat vs. eggs) is not fully comparable (Figure 4.8).

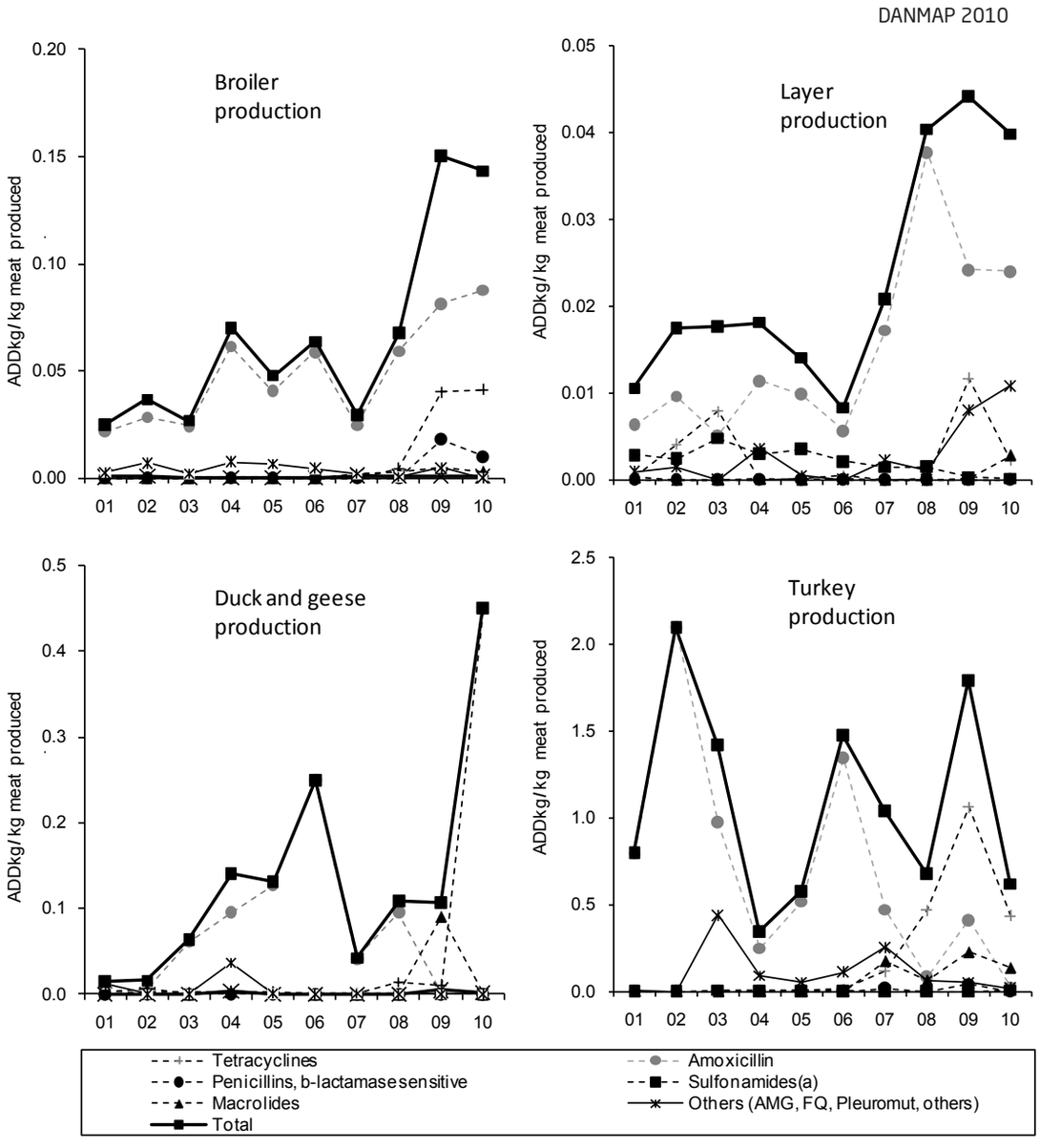
In turkeys, the annual consumption is highly variable. In 2010, the consumption again decreased to a relatively low level to a total of 252 kg or 0.62 ADDkg/kg meat produced (Table 4.3 and Figure 4.8). According to the poultry practitioners, the 2009 increase in prescription in turkeys was mainly due to *Pasteurella multocida* infections, and a vaccination campaign was conducted to control the disease in April–October 2009. Also, vaccination against haemorrhagic enteritis (viral) in turkeys was initiated in April 2010.

In 2010, tetracyclines comprised 70% of the antimicrobial consumption in turkeys, and have from 2008 been the major drug of choice. However, before 2007, amoxicillin constituted 70–99% of the consumption in turkeys, while the use had decreased to 3% in 2010. The changes in prescription occurred after the marketing of tetracyclines and other agents for use in poultry during 2007–2008. Before 2007, only amoxicillin and fluoroquinolones were marketed for poultry. Fluoroquinolones were not used in 2010, neither in the turkey production nor in *Gallus gallus*, and the consumption has decreased since 2006 when fluoroquinolones comprised 7% of the antimicrobial consumption in these species.

Measured in ADDkg per kg meat produced, the consumption in ducks and geese increased three fold in 2010, a level approximately three times higher (per kg meat produced) than in the broiler production (Figure 4.8).

Annual production data are not available for game birds. The population was estimated at 1 million pheasants, 0.5 million ducks and 0.1 million other birds in 2004. Assuming a constant population, the antimicrobial consumption in game birds has been stable during 2002–2009; around 1 ADD_{kg}/kg meat produced (Appendix 1, Table AP1.3). At least part of the apparent increase in consumption in 2010 is due to a

Figure 4.8. Consumption of antimicrobial agents in the poultry production, Denmark



bias in the previous years of reporting, because missing information of poultry species has mostly been related to the production of game birds. Thus, the increase in registered consumption for game birds is - at least in part - related to the decrease in the "species unknown" category.

4.3.4 Antimicrobial consumption in fur animals, aquaculture and pet animals

The production of fur animals included 14 million mink, 34,000 chinchillas and a minor production of foxes in 2010 (as in 2009). In 2010, the consumption in fur animals increased to 3,714 kg in 2010, representing a 16% increase compared with 2009. Please note that the data in DANMAP 2009 are not directly comparable, when including all data reported from practice, the consumption amounted to 3,200 kg in 2009. In 2010, aminopenicillins remained the most commonly used antimicrobial class in fur animals, increasing to 41% of the antimicrobial consumption (kg active substance) in 2010. Macrolides, tetracyclines and sulfonamide-trimethoprim combinations comprised another 56%. Fluoroquinolones were not used in 2010.

The antimicrobial consumption in aquaculture decreased by 7% to 3,060 kg in 2010 compared to 2009 (Figure 4.1). In aquaculture, the major class of antimicrobial was sulfonamide/trimethoprim, comprising 66% of the consumption in aquaculture. The consumption of quinolones (oxolinic acid) comprised 27% of the consumption in aquaculture in 2010, and has increased continuously since 2007. The overall decrease was mainly due a change in choice of antimicrobial agents towards oxolinic acid (10 mg/kg), which has a much lower dosage than sulfonamide-trimethoprim (30 mg/kg). The consumption in salt water fish was

9 ADD_{kg}/kg fish produced in 2009 and 2010. The antimicrobial consumption in salt water aquaculture peaked in 2006, reaching 13 ADDkg/kg fish produced, due to an unusually warm summer. Fish production is very sensitive to water temperatures, but also increasing vaccination intensity has caused a gradual 51% decrease in antimicrobial use in salt water aquaculture through 2006–2010 [personal communication: N.H. Henriksen, Danish Aquaculture]. Regarding fish produced in fresh water, the consumption is more stable around 2 ADDkg /kg fish produced; the consumption increased by 8% to ADDkg/kg fish produced from 1.8 ADDkg/kg in 2009, to 2.0 ADDkg/kg in 2010, when assuming the same production volume as in 2009. The increase was most likely related to a failure in vaccine deliveries during winter 2009–2010 [personal communication: N.H. Henriksen, Danish Aquaculture].

The consumption of antimicrobial agents in companion animals (pet animals and horses) was 3 tonnes, estimated from the prescription for these species and sales for companion animal practices. This is higher than the estimated 2.2 tonnes in 2009, but this is partly due to an underestimation in previous years. Looking at oral medicines for pet animals only, the prescription did not increase from 2009 to 2010, however, during the past decade the veterinary prescription of oral medicines increased by 24%. The major antimicrobial agent used in pet animals, amoxicillin in combination with clavulanic acid, increased by 3% to 539 kg in 2010 compared to 2009, as part of a continuous increase over the past decade. Other frequently used drugs were cephalosporins (317 kg), mainly first generation for oral use, representing a 9% decrease compared to 2009. In pet animals, the consumption of 3rd and 4th generation cephalosporin was an estimated 3 kg, corresponding to 1.8% of the total veterinary consumption of these antimicrobials. The use of fluoroquinolones in pet animals was estimated 14 kg in 2010 and this corresponded to 72% of the total veterinary use of fluoroquinolones in Denmark.

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ANTIMICROBIAL CONSUMPTION IN HUMANS 5

5. Antimicrobial consumptions in humans

5.1 Introduction

All systemic antimicrobial agents used in Denmark are prescription medications only, and all Danish medical doctors have the right to prescribe freely what they find appropriate for their patients. There are no restrictions in the prescribing of antibacterial agents. National guidelines exist for the prudent use of antibacterial agents, as well as local guidelines from each of the Departments of Clinical Microbiology (DCM); taking into account the local resistance patterns.
Throughout the 'Antimicrobial consumption in humans' section, the consumption of 2010 is compared with the consumption of 2009 and of the last decade (2001), respectively. Also, combinations of penicillins, incl. beta-lactamase inhibitors (J01CR) are referred to as 'combination penicillins'.
Antibacterial agents used for systemic treatment in humans (and in animals) are listed in Table 3.2.

Narrow- & Broad-spectrum Agents. Antibacterial agents have been classified as either narrow-spectrum or broad-spectrum agents according to the width of the activity against Gram-positive and Gram-negative bacteria (Table 5.1).

Defined Daily Dose (DDD). The DDD is the assumed average maintenance dose per day for a drug used for its main indication in adults. It should be emphasised that DDD is a unit of measurement and does not necessarily reflect the recommended or prescribed daily dose [http://www.whoc.no].

DDD per 1,000 inhabitants per day (DID). Consumption in primary health care is presented in DID. Also, consumption in hospital care (rehabilitation centres, hospices, private-, psychiatric-, specialised-, and somatic hospitals) and the merged total consumption is presented in DID enabling the comparison of the two sectors, and illustrating the consumption in hospital care without the activity in

Table 5.1. Classification of antibacterial agents for systemic use in humans into narrow-spectrum and broad-spectrum agents, Denmark

DANMAP 2010

ATC group[a]	Therapeutic group
	Narrow-spectrum
J01CE	Beta-lactamase sensitive penicillins
J01CF	Beta-lactamase resistant penicillins
J01DB	First-generation cephalosporins (included in data from primary health care as a broad-spectrum agent in the group J01D)
J01DF	Monobactams
J01EA	Trimethoprim and derivatives
J01EB	Short-acting sulfonamides
J01FA	Macrolides
J01FF	Lincosamides
J01XA	Glycopeptides
J01XC	Steroid antibacterials (fusidic acid)
J01XD	Imidazol derivatives
J01XE	Nitrofuran derivatives (nitrofurantoin)
J01XX	Other antibacterials
	Broad-spectrum
J01AA	Tetracyclines
J01CA	Penicillins with extended spectrum
J01CR	Combinations of penicillins, incl. beta-lactamase inhibitors
J01D	Cephalosporins and related substances (primary health care only)
J01DC	Second-generation cephalosporins
J01DD	Third-generation cephalosporins
J01DH	Carbapenems
J01EE	Combinations of sulfonamides and trimethoprim, incl. derivatives
J01GB	Aminoglycosides
J01MA	Fluoroquinolones
J01XB	Polymyxins

a) From the 2010 edition of the Anatomical Therapeutic Chemical (ATC) classification system

the hospitals. Data presented in DID provide a rough estimate of the proportion of the population within a defined area treated daily with certain drugs. For example, 10 DIDs indicates that 1% of the population on average gets a certain treatment daily.

Packages per 1,000 inhabitants per year. Assuming that one package is prescribed for one prescription, packages per 1,000 inhabitants per year serve as a surrogate for prescriptions or treatments given to the primary health care population.

Treated patients per 1,000 inhabitants per year. Illustrates the number of patients treated in primary health care.

Kilogram. To allow comparison with consumption of antibacterial agents in animals, total human consumption is also presented in kilograms.

DDD per 100 occupied bed-days (DBD) and DDD per 100 admissions (DAD). Consumption in somatic hospitals is presented in both DBD and DAD to include the activity in hospitals. DAD is introduced in this report, since it is the internationally recognised abbreviation. It is the same measure as DDD per 100 discharges (discharged patients) which has been used in the previous DANMAP reports.

5.2 Total consumption of both primary health care and hospital care

Total consumption compared with 2009

In 2010, the total consumption of antibacterial agents for systemic use (primary health care and hospital care) increased by 5%: from 17.89 DDDs per 1,000 inhabitants per day (DID) in 2009 to 18.84 DID in 2010 (Figure 5.1). The increase was noticed in primary health care only, whereas the consumption in hospital care was similar to the year before. Broad-spectrum agents increased by 7%; representing 7.76 DID of the total consumption in 2010 compared with 7.24 DID in 2009 (Figure 5.2). The percentage of DDDs prescribed in primary health care represented 90% of the total human consumption.

Figure 5.3 shows the distribution of the total number of DIDs of antibacterial agents between primary health care and hospital care. For example, sulfonamides and trimethoprim (J01E) and beta-lactamase resistant penicillins (J01CF) had a ratio of consumption in primary health care vs. consumption in hospital care of around 9/1 and 6/1, respectively.

Total consumption - the last decade

Since 2001, consumption has increased by 4.54 DID (32%) (Figure 5.1). Also, broad-spectrum agents have increased by 3.3 DID (74%); comprising 41% of the total consumption in 2010 compared with 31% in 2001 (Figure 5.2). During the last decade, the proportion of DDDs prescribed in primary health care represented 89–90% of the total human consumption.

To view the detailed distribution of DIDs among antibacterial groups in primary health care and hospital care, please refer to Table 5.3 and Table AP1.4 in Appendix 1, respectively.

Total consumption in kilograms

In 2010, 50.67 tonnes of antibacterial agents for systemic use were used in humans in Denmark representing an increase of 2.06 tonnes (4%) compared with 2009, and an increase of 8.67 tonnes (21%) compared with 2001 (Table 5.2).

Figure 5.1. Total consumption of antibacterial agents (J01) in humans by sector, Denmark

DANMAP 2010

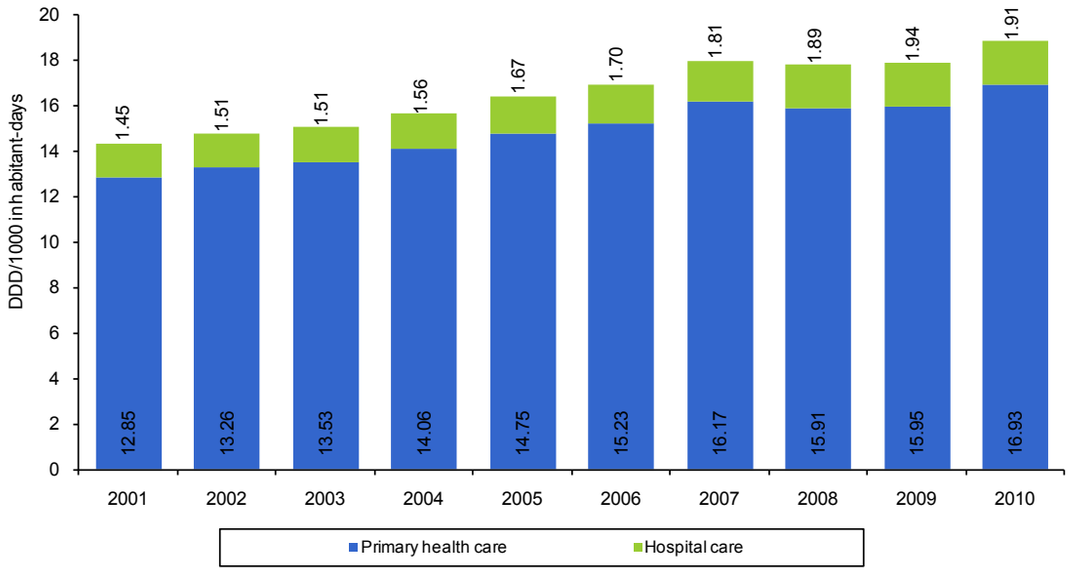
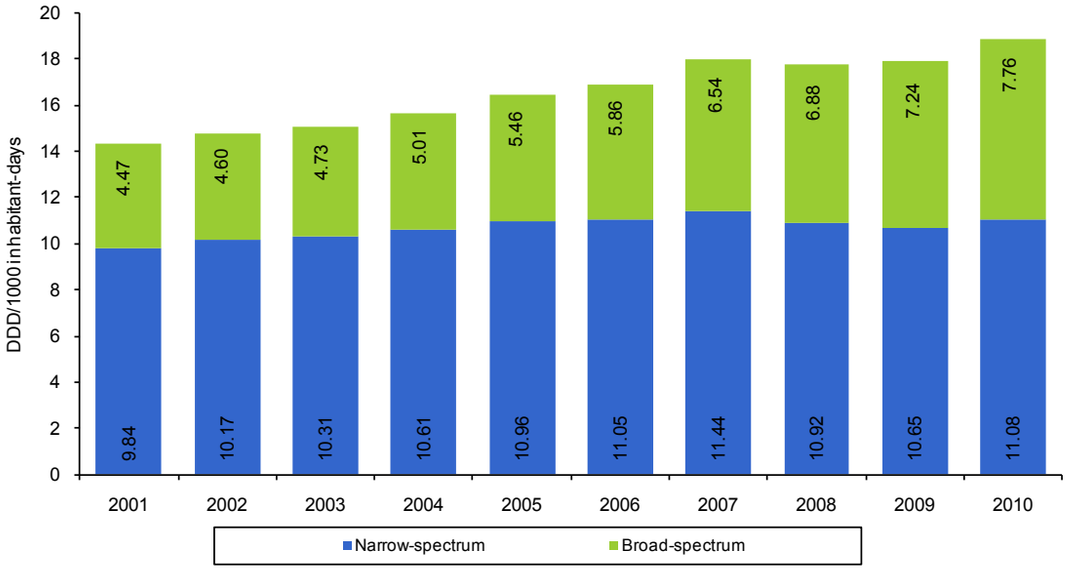


Figure 5.2. Total consumption of antibacterial agents (J01) in humans by narrow-spectrum[a] and broad-spectrum[b] agents, Denmark

DANMAP 2010



a) Narrow-spectrum includes: beta-lactamase sensitive penicillins, beta-lactamase resistant penicillins, trimethoprim, sulfonamides, macrolides, lincosamides, glycopeptides, fusidic acid, imidazol derivatives, nitrofuran derivatives and 'other antibiotics'
b) Broad-spectrum includes: tetracyclines, penicillins with extended spectrum, combinations of penicillins incl. beta-lactamase inhibitors, cephalosporins and related substances, combinations of sulfonamides and trimethoprim, aminoglycosides, fluoroquinolones and polymyxins

Figure 5.3. Distribution of DIDs between primary health care and hospital care, Denmark

DANMAP 2010

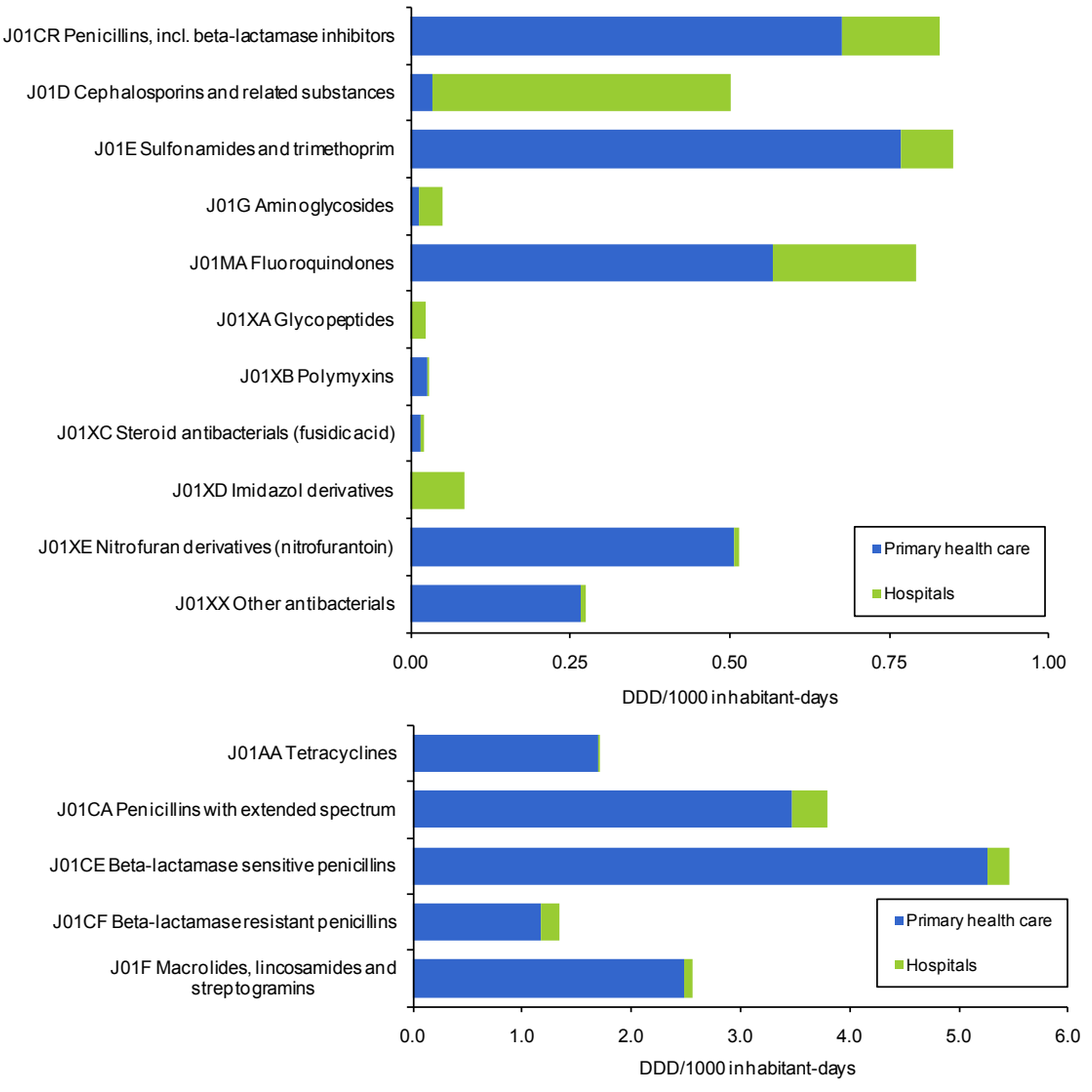


Table 5.2. Consumption of antibacterial agents for systemic use in humans (kg active substance), Denmark

DANMAP 2010

ATC group[a]	Therapeutic group	Year									
		2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
J01AA	Tetracyclines	1475	1501	1542	1636	1748	1835	1855	1884	2039	2161
J01B	Amphenicols	1	0	0	0	0	0	0	0	0	0
J01CA	Penicillins with extended spectrum	5385	5356	5295	5346	5561	5722	6188	6061	6076	6317
J01CE	Beta-lactamase sensitive penicillins	20730	21263	21630	22230	22520	22760	24003	22466	21744	22301
J01CF	Beta-lactamase resistant penicillins	3230	3738	4075	4377	4565	4842	5037	5183	5250	5418
J01CR	Comb. of penicillins, including beta-lactamase inhibitors	146	249	336	480	534	724	1012	1348	1836	2597
J01D	Cephalosporins and related substances[b]	739	811	830	894	1582	1778	2285	2530	2740	2696
J01EA	Trimethoprim and derivatives	280	293	307	334	359	382	402	402	399	417
J01EB	Short-acting sulfonamides	3113	3092	3064	3067	2987	2865	2565	2273	2200	2158
J01EE	Comb. of sulfonamides and trimethoprim, including derivatives	289	288	273	185	208	208	148	183	193	252
J01FA	Macrolides[c]	4089	4150	3876	3743	3775	3542	3434	3164	2966	3038
J01FF	Lincosamides[b]	37	40	45	53	52	66	78	94	113	124
J01G	Aminoglycosides	30	31	28	31	31	27	27	25	23	24
J01MA	Fluoroquinolones[b]	398	451	611	722	866	979	1162	1351	1371	1457
J01XA	Glycopeptides	36	42	43	46	51	56	61	64	86	89
J01XC	Steroid antibacterials (fusidic acid)	59	59	58	52	62	65	67	64	62	65
J01XD	Imidazoles	168	179	191	195	206	198	202	241	255	258
J01XE	Nitrofuran derivatives (nitrofurantoin)	155	163	166	171	180	185	190	192	201	208
J01XX05	Methenamine[b]	1637	1662	1590	1473	1107	1076	1060	1087	1047	1078
J01XX08+09	Linezolid, daptomycin	0	3	4	5	10	14	12	14	14	13
J01	Antibacterial agents for systemic use (total)[d]	41997	43371	43964	45040	46404	47324	49788	48629	48614	50673

Note: Includes data from both primary health care and hospital care and has been recalculated from original data expressed as DDDs. For monitoring in human primary health care and hospital care, the recommended way of expressing consumption is DDDs per 1000 inhabitant-days and DDDs per 100 occupied bed-days / DDDs per 100 admissions (see Tables 5.3, 5.5 and 5.6)

a) From the 2010 edition of the ATC classification system

b) Since 2005, the kg active substance was estimated taking into account the DDD for each route of administration, e.g. cefuroxime parenteral DDD = 3 g and cefuroxime oral DDD = 0.5 g. From 2001 to 2004, it was estimated with a DDD corresponding to an average for the various routes, e.g. for cefuroxime: 1.75 g

c) When two different DDDs of an antimicrobial agent existed for different presentations, an average DDD was used. Estimates using the lowest and the highest calculated limit are 2473–3603 for 2010

d) Does not include polymyxins

5.3. Primary health care

5.3.1 Total consumption in primary health care

Total consumption compared with 2009

In 2010, the total consumption of antibacterial agents for systemic use (J01) in primary health care increased by 6% to 16.93 DID compared with 15.95 DID in 2009, and increases were observed for 10/19 of the therapeutic groups (Table 5.3). Four therapeutic groups constituted most of the increase: macrolides (0.23 DID); 'combination penicillins' (0.23 DID); penicillins with extended spectrum (0.18 DID); and beta-lactamase sensitive penicillins (0.13 DID). Consumption decreased within only one group: short-acting sulfonamides 0.01 DID (2%).

The increase this year of 0.98 DID (6%) was the largest increase observed, since the DANMAP programme was initiated in 1995. Each treated patient, in primary health care, used almost equal numbers of DDD compared with 2009 (19.6 DDD vs. 19.2 DDD), as demonstrated in paragraph 5.3.2. However, more patients were treated in 2010 with additional packages prescribed (Tables AP1.4, AP1.5 in appendix 1). The fact that more patients were treated in 2010 - at least once by an antibacterial agent with an equal number of DDDs per patient - has led to the increase in consumption.

The observed increase in consumption was markedly larger in the second half of 2010, both for total consumption (J01), but also for macrolides and beta-lactamase sensitive penicillins. This coincided with an increased burden of lower respiratory tract infections (LRTI) in the second half of 2010, confirmed by an outbreak of *Mycoplasma pneumoniae* in the (third

Table 5.3. Consumption of antibacterial agents for systemic use in primary health care (DDD/1000 inhabitant-days), Denmark

DANMAP 2010

ATC group(a)	Therapeutic group	Year									
		2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
J01AA	Tetracyclines	0.99	1.04	1.07	1.17	1.28	1.38	1.48	1.54	1.61	1.69
J01CA	Penicillins with extended spectrum	2.47	2.51	2.52	2.63	2.79	2.95	3.25	3.26	3.29	3.47
J01CE	Beta-lactamase sensitive penicillins	4.91	5.00	5.07	5.20	5.28	5.40	5.67	5.30	5.12	5.25
J01CF	Beta-lactamase resistant penicillins	0.65	0.77	0.85	0.92	0.97	1.05	1.09	1.12	1.13	1.17
J01CR	Combinations of penicillins, including beta-lactamase inhibitors	0.03	0.04	0.05	0.06	0.08	0.12	0.19	0.27	0.45	0.68
J01D	Cephalosporins and related substances	0.03	0.03	0.02	0.02	0.03	0.03	0.03	0.03	0.03	0.03
J01EA	Trimethoprim and derivatives	0.35	0.36	0.38	0.41	0.44	0.47	0.49	0.49	0.48	0.51
J01EB	Short-acting sulfonamides	0.36	0.36	0.36	0.36	0.35	0.35	0.31	0.28	0.27	0.26
J01EE	Combinations of sulfonamides and trimethoprim, including derivatives	0.04	0.03	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00
J01FA	Macrolides	2.10	2.15	2.13	2.23	2.41	2.31	2.42	2.28	2.21	2.44
J01FF	Lincosamides	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.03	0.03	0.04
J01GB	Aminoglycosides	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
J01MA	Fluoroquinolones	0.17	0.18	0.25	0.28	0.33	0.37	0.44	0.51	0.52	0.57
J01XA	Glycopeptides	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
J01XB	Polymyxins	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
J01XC	Steroid antibacterials (fusidic acid)	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.01	0.01
J01XE	Nitrofuran derivatives (nitrofurantoin)	0.39	0.41	0.42	0.43	0.45	0.46	0.47	0.47	0.49	0.51
J01XX	Other antibacterials (methenamine >99%)	0.33	0.34	0.32	0.30	0.28	0.27	0.26	0.27	0.26	0.27
J01	Antibacterial agents for systemic use (total)	12.85	13.26	13.53	14.06	14.75	15.23	16.17	15.91	15.95	16.93

a) From the 2010 edition of the Anatomical Therapeutic Chemical (ATC) classification system

and) fourth quarter of 2010 (Figure 5.11). LRTI's of suspected bacteriological origin are empirically treated with beta-lactamase sensitive penicillins, and confirmed *M. pneumoniae* pneumonia by macrolides according to guidelines in Denmark. Therefore, it is a likely conclusion that the *M. pneumoniae* pneumonia outbreak explains a good part of the increased consumption. Unfortunately, indication codes are incomplete, and therefore this presumption cannot be verified.

Another important factor was the increased consumption of 'combination penicillins', contributing 23% of the total increase. In this therapeutic group, indeed more patients were treated in 2010 with additional packages pre-scribed (Tables AP1.4 and AP1.5 in appendix 1), and additional DDD's were given to each treated patient (Table 5.4). For possible explanations to this increase; refer to paragraph 5.3.2.

Beta-lactamase sensitive penicillins still represented the largest therapeutic group of antibacterial agents consumed (31%) followed by penicillins with extended spectrum (20%) and macrolides (15%) (Figure 5.4). Penicil-lins (J01C) accounted for 62% of the total consumption in 2010. Consumption of broad-spectrum agents increased by 0.53 DID (9%) compared with 2009 (Figure 5.5).

Total consumption - the last decade

Antibacterial consumption (J01) increased by 32% from 12.85 DID in 2001 to 16.93 DID in 2010 (Table 5.3). Broad-spectrum agents represented 6.48 DID (38%) of the total consumption in 2010 compared with 3.75 DID (29%) in 2001; representing an increase of 73% (Figure 5.5). For all leading groups of antibacterial agents (tetracyclines, penicillins with extended spectrum, beta-lactamase sensitive penicillins, beta-lactamase resistant penicillins, 'combination penicillins', macrolides, and fluoroquinolones) consumption was higher in 2010 than 10 years before, and for most groups the trend in consumption has been a steady increase year by year (Figure 5.6). Only short-acting sulfonamides (J01EB) and 'other antibiotics' (J01XX) were at a lower reported level in 2010 compared with 2001.

5.3.2 Measures at treated patient level

Measures at treated patient level compared with 2009

The total number (J01) of DDDs per treated patient was 19.6 in 2010 compared with 19.2 in 2009. Among substances with the highest consumption (DID), each treated patient received from 11–21 DDDs in 1.4–1.6 packages with the exception of tetracyclines (45.2

Figure 5.4. Distribution of the total consumption of antibacterial agents in primary health care, Denmark

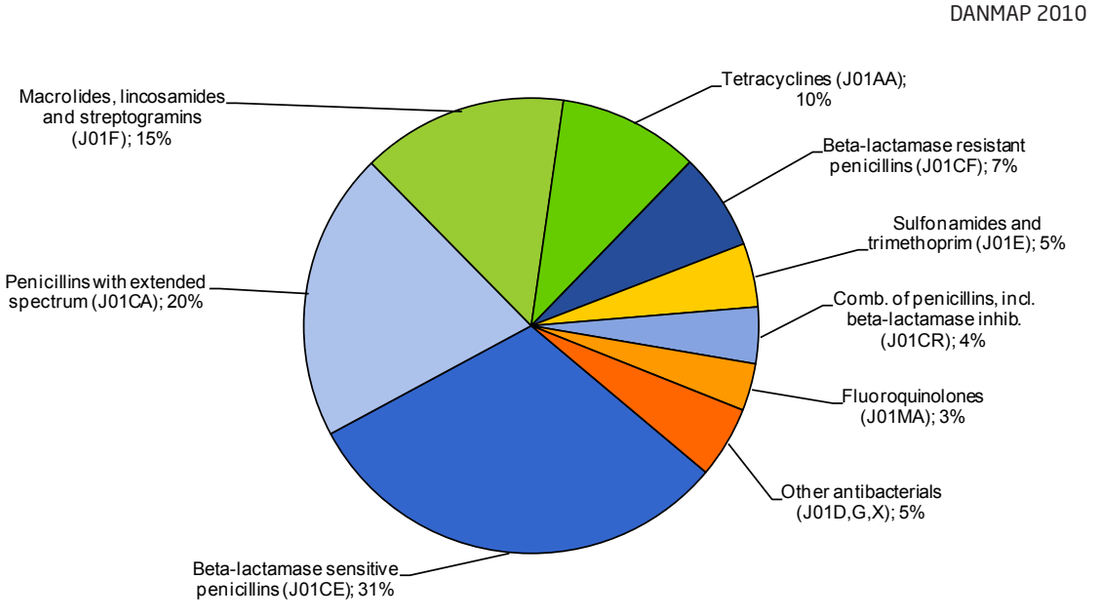
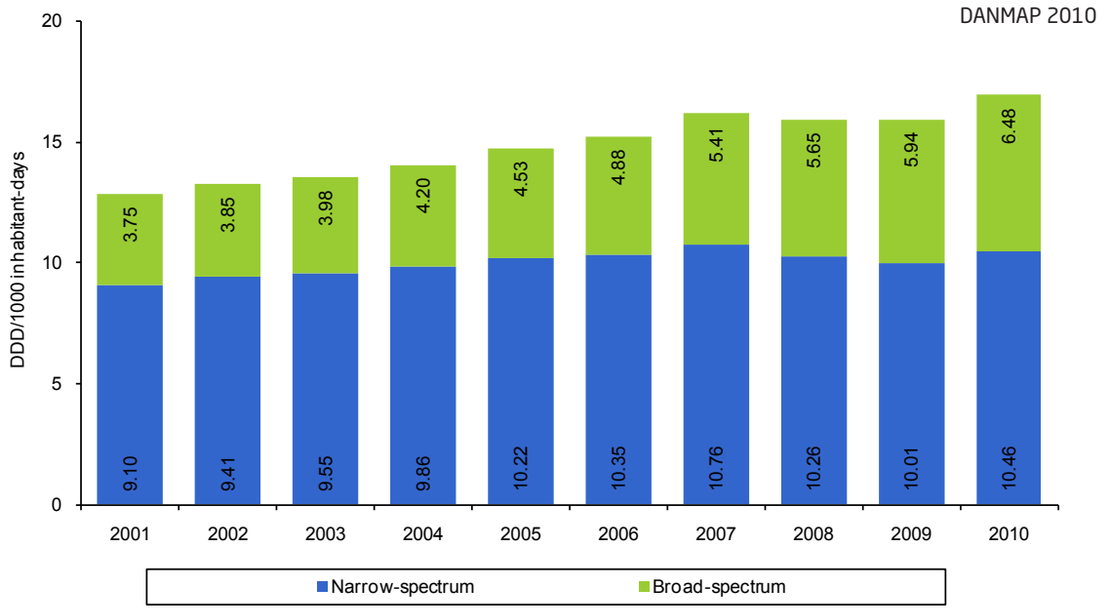
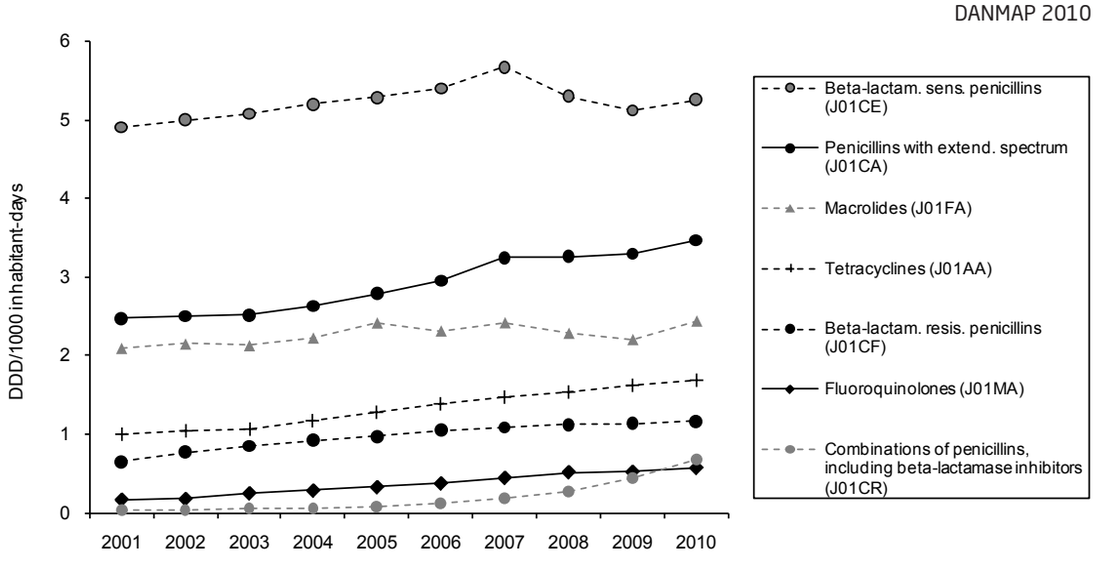


Figure 5.5. Consumption of antibacterial agents (J01) in primary health care by narrow-spectrum[(a)] and broad-spectrum[(b)] agents, Denmark



a) Narrow-spectrum includes: beta-lactamase sensitive penicillins, beta-lactamase resistant penicillins, trimethoprim, sulfonamides, macrolides, lincosamides, glycopeptides, fusidic acid, imidazol derivatives, nitrofuran derivatives, and 'other antibiotics'
b) Broad-spectrum includes: tetracyclines, penicillins with extended spectrum, combinations of penicillins incl. beta-lactamase inhibitors, cephalosporins and related substances, combinations of sulfonamides and trimethoprim, aminoglycosides, fluoroquinolones and polymyxins

Figure 5.6. Consumption of leading antibacterial groups for systemic use in primary health care, Denmark



DDDs in 2.0 packages) (Table 5.4 and Table AP1.6 in appendix 1).

Measures at treated patient level - the last decade
Different indicators of antibacterial consumption at treated patient level in primary health care are available (Figure 5.7). Comparing 2001–2010, DDD's have been the indicator increasing the most; both as an increasing number of DDDs per treated patient (25%) and as DDDs per prescribed package (20%) (Table 5.4). Tetracyclines, 'combination penicillins', and fluoroquinolones have shown the largest increases.

The nature of these changes in trends found over the last decade are not clear, mainly because codes of indication are incomplete as pointed out in Textbox 3, DANMAP 2008. A list of the changes in packages and guidelines that have occurred during the last decade that could help to explain these trends is displayed below.

Tetracyclines: the available packages (size and strength) have not been altered over the last decade. Prescriptions of packages with higher numbers of tablets could be explanatory for this trend as pointed out in DANMAP 2008.

Penicillins with extended spectrum: a few low-strength packages (for children) are no longer marketed (higher strength packages are prescribed for small children) and national guidelines have increased the recommended dosage of certain substances over the last decade [DANMAP 2008].

Beta-lactamase sensitive penicillins: some low-strength packages (for children) are no longer marketed while packages with higher strength have been introduced (higher strength packages are prescribed for small children); recommended dosage has increased for certain indications over the last decade [DANMAP 2008].

Beta-lactamase resistant penicillins: no changes in packages have occurred over the last decade, but national guidelines have introduced new indications of treatment for certain infections (mastitis and impetigo) over the last decade [DANMAP 2008].

Table 5.4. Number of DDDs and packages per treated patient among leading groups of antibacterial agents in primary health care, Denmark

DANMAP 2010

ATC group[a]	Therapeutic group	Indicator	Year									
			2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
J01AA	Tetracyclines	DDDs / patient	30.6	33.0	34.4	36.9	39.0	40.9	43.0	44.4	45.2	45.9
		Packages / patient	1.9	1.9	1.9	1.9	2.0	1.9	2.0	2.0	2.0	2.0
		DDDs / package	16.1	17.5	18.1	19.0	19.6	21.0	22.0	22.7	22.7	22.7
J01CA	Penicillins with extended spectrum	DDDs / patient	13.0	13.2	13.4	13.6	13.9	14.2	14.4	14.7	14.8	14.9
		Packages / patient	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6
		DDDs / package	8.1	8.2	8.2	8.4	8.5	8.9	9.0	9.2	9.2	9.0
J01CE	Beta-lactamase sensitive penicillins	DDDs / patient	10.3	10.5	10.7	11.1	11.3	11.5	11.7	11.8	11.8	11.8
		Packages / patient	1.4	1.5	1.5	1.5	1.5	1.4	1.4	1.4	1.4	1.4
		DDDs / package	7.1	7.2	7.3	7.5	7.7	8.0	8.2	8.2	8.4	8.4
J01CF	Beta-lactamase resistant penicillins	DDDs / patient	12.4	11.8	11.8	12.4	12.7	13.0	13.4	13.7	13.9	14.2
		Packages / patient	1.6	1.6	1.6	1.6	1.6	1.5	1.5	1.5	1.5	1.5
		DDDs / package	7.9	7.5	7.4	7.8	8.0	8.6	8.7	9.0	9.1	9.3
J01CR	Combinations of penicillins, incl. beta-lactamase inhibitors	DDDs / patient	15.9	14.7	16.6	17.2	16.8	19.3	19.1	19.9	20.4	21.1
		Packages / patient	1.7	1.7	1.8	2.0	2.0	1.8	1.6	1.6	1.5	1.5
		DDDs / package	9.1	8.6	9.1	9.1	9.3	10.7	11.7	12.4	13.3	13.7
J01FA	Macrolides	DDDs / patient	11.3	11.7	12.1	12.4	12.4	12.6	12.4	12.5	12.5	12.2
		Packages / patient	1.5	1.5	1.6	1.6	1.6	1.5	1.5	1.5	1.5	1.5
		DDDs / package	7.5	7.6	7.8	7.9	8.0	8.3	8.1	8.1	8.1	8.1
J01MA	Fluoroquinolones	DDDs / patient	8.3	8.6	10.3	9.5	9.6	10.3	10.6	11.0	11.2	11.2
		Packages / patient	1.4	1.4	1.6	1.5	1.5	1.5	1.5	1.5	1.5	1.5
		DDDs / package	5.9	6.0	6.6	6.4	6.5	6.9	7.0	7.5	7.6	7.6
J01	Antibacterial agents for systemic use (total)	DDDs / patient	15.6	16.0	16.4	17.0	17.5	17.9	17.3	18.9	19.2	19.6
		Packages / patient	2.0	2.0	2.1	2.1	2.1	2.0	1.9	2.1	2.1	2.1
		DDDs / package	7.8	7.8	7.9	8.1	8.3	8.7	8.9	9.1	9.3	9.3

a) From the 2010 edition of the Anatomical Therapeutic Chemical (ATC) classification system

'Combination penicillins': the proportion of children (<15 years) receiving these substances have decreased and been replaced by adults as pointed out in the DANMAP 2007 report. As a consequence, the lowest strength packages are no longer marketed and low-strength packages have presumably been replaced by packages with higher strength. Also, national guidelines have introduced amoxicillin and clavulanic acid (J01CR02) as first-choice drug for treatment requiring acute exacerbation of chronic obstructive pulmonary disease, and for the treatment of recurrent/persistent upper respiratory tract infections (2007 guideline). In fact, whereas 28% of the consumption was dispensed as mixture (for children) in 2001, the fraction was only 5% in 2010.

Macrolides: the packages have not changes over the last decade, but national guidelines have increased the recommended treatment dosage for certain indications over the last decade [DANMAP 2008].

Fluoroquinolones: National guidelines only recommend these substances as second line choices for a limited number of indications, and no changes in packages have occurred over the last decade that could explain the trends in consumption.

5.3.3 Tetracyclines (J01AA)

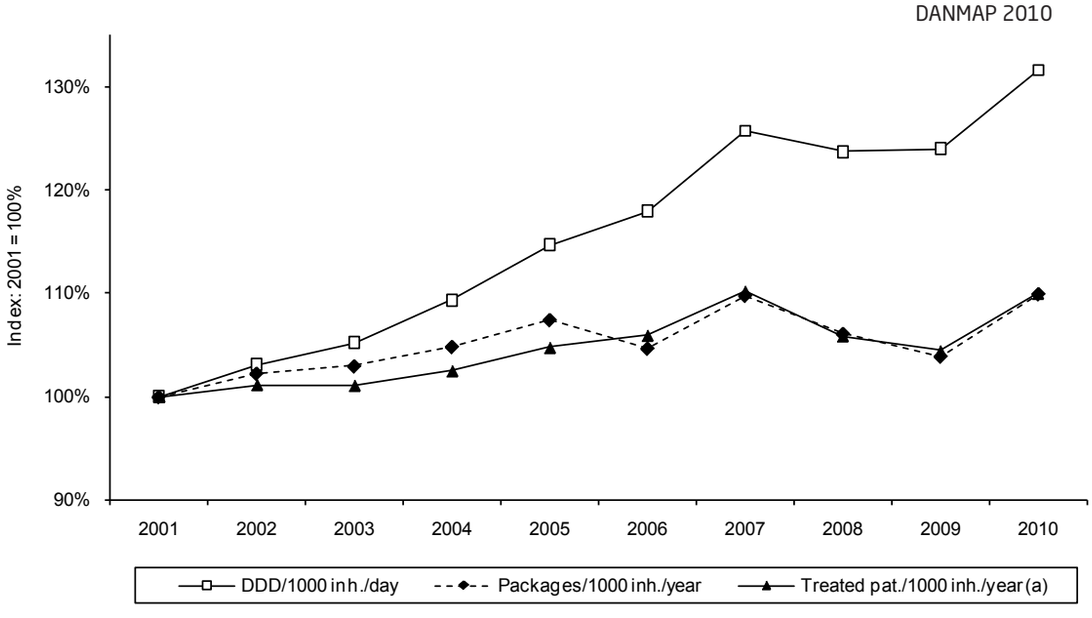
Consumption of tetracyclines (J01AA) compared with 2009

In 2010, consumption of tetracyclines increased by 0.08 DID (5%) compared with 2009 (Table 5.3). Tetracycline (0.71 DID (42%)) was the most used of the tetracyclines in 2010 followed by doxycycline (0.55 DID (33%)), lymecycline (0.36 DID (21%)) and oxytetracycline (0.07 DID (4%)), respectively (Figure 5.8). Within the group, consumption of all substances increased in 2010 compared with 2009.

Consumption of tetracyclines (J01AA) - the last decade

Since 2001, an extensive increase in the consumption of tetracyclines (0.70 DID (70%)) has been observed (Table 5.3). As previously pointed out in the DANMAP 2007 report, a large part of the consumption of tetracyclines is prescribed for teenagers and young adults, and a binary pattern of consumption has been observed (tetracycline and lymecycline with peak

Figure 5.7. Indicators of antibacterial consumption (J01) in primary health care, Denmark



a) Cumulated number of patients treated with anbacterials (ATC-4 level). The Danish Medicines Agency counts the first treatment within each ATC-group for each patient, each year

values in the spring and autumn, doxycycline with peak values in January and June). Also, 43% of the tetracycline (J01AA07) consumption is known to be prescribed against acne, and 9% of the doxycycline (J01AA02) consumption as malaria prophylaxis [Textbox 3 DANMAP 2008]. However, a total understanding of the increased consumption is not possible, as codes of indication are incomplete.

5.3.4 Penicillins (J01C)

Consumption of penicillins (J01C) compared with 2009

In 2010, consumption of penicillins increased by 0.57 DID (6%) compared with 2009. The four main groups: penicillins with extended spectrum, beta-lactamase sensitive penicillins, beta-lactamase resistant penicillins, and 'combination penicillins' all increased (Table 5.3). The only penicillin that did not increase in 2010 was pivampicillin (J01CA02) (Figure 5.9).

Consumption of penicillins (J01C) - the last decade

The decreasing consumption of phenoxymethylpenicillin and amoxicillin seen in 2009 and 2008 was not continued in 2010, but a new plateau may have been reached (Figure 5.9). This lowered level of consumption could be the result of a decreased disease burden after the introduction of a heptavalent conjugate pneumococcal vaccine (PCV7) in the Danish Childhood Immunization Programme in October 2007, but this has not been documented. Recently, a report has demonstrated that the incidence of invasive pneumococcal disease has dropped from 20 cases / 100,000 inhabitants (1,055 cases per year on average) in the years prior to the introduction of PCV7 to 18 cases / 100,000 inhabitants (982 cases per year on average) in the years after the introduction of PCV7 [EPI-NYT 19, 2011].

Over the last decade (2001–2010), the consumption of penicillins with extended spectrum has increased by 1.00 DID (40%), beta-lactamase sensitive penicillins has increased by 0.35 DID (7%), beta-lactamase

Figure 5.8. Consumption of tetracyclines in primary health care, Denmark

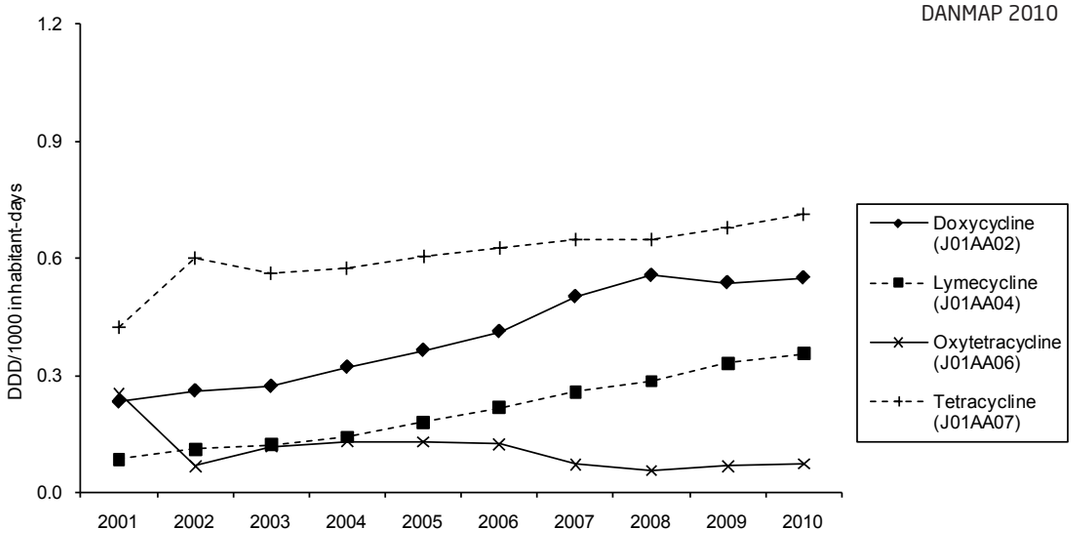
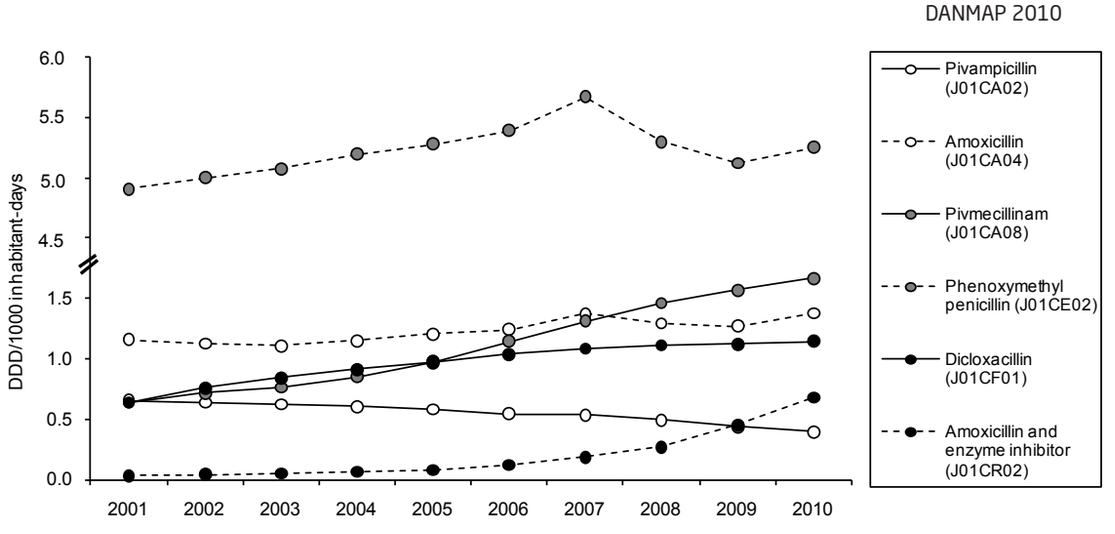


Figure 5.9. Consumption of leading penicillins in primary health care, Denmark



resistant penicillins has increased by 0.52 DID (80%) and 'combination penicillins' has increased by 0.65 DID (2277%), respectively (Table 5.3). Phenoxymethylpenicillin was still by far the most consumed penicillin, but among the other penicillins the order has changed during the last decade (Figure 5.9).

5.3.5 Macrolides (J01FA)

Consumption of macrolides (J01FA) compared with 2009

The consumption of macrolides increased by 0.23 DID (10%) from 2009–2010 (Table 5.3). Within the group of macrolides, clarithromycin (0.03 DID), azithromycin (0.04 DID) and erythromycin (0.04 DID) consumption fol-lowed the trends of the previous years whereas roxithromycin showed a considerable increase of 0.19 DID (Figure 5.10). As in 2004 and 2005 [Figure 12, DANMAP 2005], part of the increase in roxithromycin consumption was likely due to an outbreak of *Mycoplasma pneumoniae* in the (third and) fourth quarter of 2010 [Rasmussen *et al.* 2010. Euro Surveill. 15. pii: 19708] (Figure 5.11). In fact, macrolide consumption was 0.91 DID (40%) higher in the fourth quarter of 2010 compared with the fourth quarter of 2009.

Consumption of macrolides (J01FA) - the last decade

Over the last decade (2001–2010), roxithromycin (0.79 DID) consumption, and to some extend clarithromycin (0.07 DID) and azithromycin (0.02 DID) consumption, has increased while erythromycin (0.54 DID) consumption has decreased (Figure 5.10). National guidelines regarding first-choice macrolide in primary health care have changed from erythromycin towards first roxithromycin (2004 guideline) and subsequently

Figure 5.10. Consumption of macrolides in primary health care, Denmark

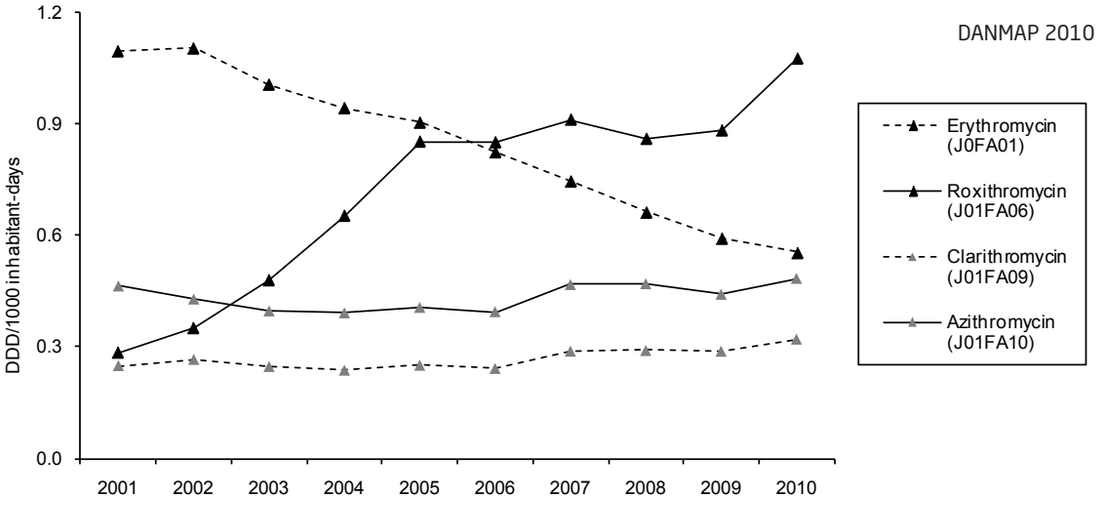
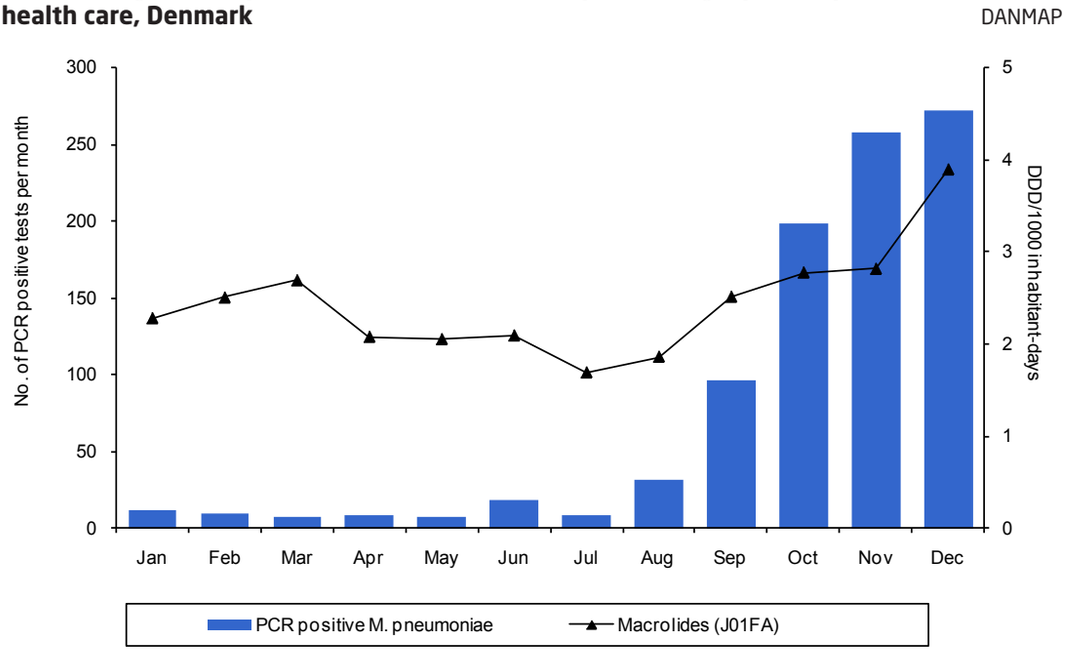


Figure 5.11. Monthly consumption of macrolides and PCR positive *Mycoplasma pneumoniae* tests in primary health care, Denmark



clarithromycin (2007 guideline) [DANMAP 2008], but the latter change has not become apparent in the distribution of the consumption. Throughout the last decade, azithromycin has been recommended for urethritis/cervicitis and epididymitis.

5.3.6 Fluoroquinolones (J01MA)

Consumption of fluoroquinolones (J01MA) compared with 2009

Fluoroquinolone consumption increased by 0.05 DID (10%) compared with 2009 (Table 5.3). Ciprofloxacin accounted for 94% of the total fluoroquinolone consumption in 2010; ofloxacin and moxifloxacin each accounted for 2% and 3%, respectively (Figure 5.12).

Consumption of fluoroquinolones (J01MA) - the last decade

Even though the trend of increasing fluoroquinolone consumption seemed to slow down in 2009, consumption has increased by 0.40 DID (238%) during 2001–2010 (Table 5.3). The continuously increasing consumption of ciprofloxacin began when the price of ciprofloxacin dropped markedly following the introduction of generics onto the Danish marked in December 2001 [Jensen *et al.* 2010. J Antimicrob Chemother. 65:1286–91].

5.4. Hospital care

5.4.1 Introduction

Hospital consumption is presented as both DDD per 100 occupied bed-days (DBD) and DDD per 100 admissions (DAD) to include the activity in hospitals, and as DID to compare with primary health care and to document the consumption in the entire hospital care sector without considering the activity in the hospitals. DAD is the internationally recognised abbreviation and is the same measure as DDD per 100 discharges which has been used in the previous DANMAP reports.

Hospital care is the term for all hospitals in the hospital care sector of Denmark i.e. rehabilitation centres, hospices, private-, psychiatric-, specialised-, and somatic hospitals. The majority of antibacterial consumption occurs in the somatic hospitals (97% of the total consumption in hospital care). Antibiotic consumption is therefore correlated to bed-days in and admissions to somatic hospitals only, and not to the number of bed-days in and admissions in all hospital care, since psychiatric hospitals contribute a large proportion of bed-days and admissions to all hospital care.

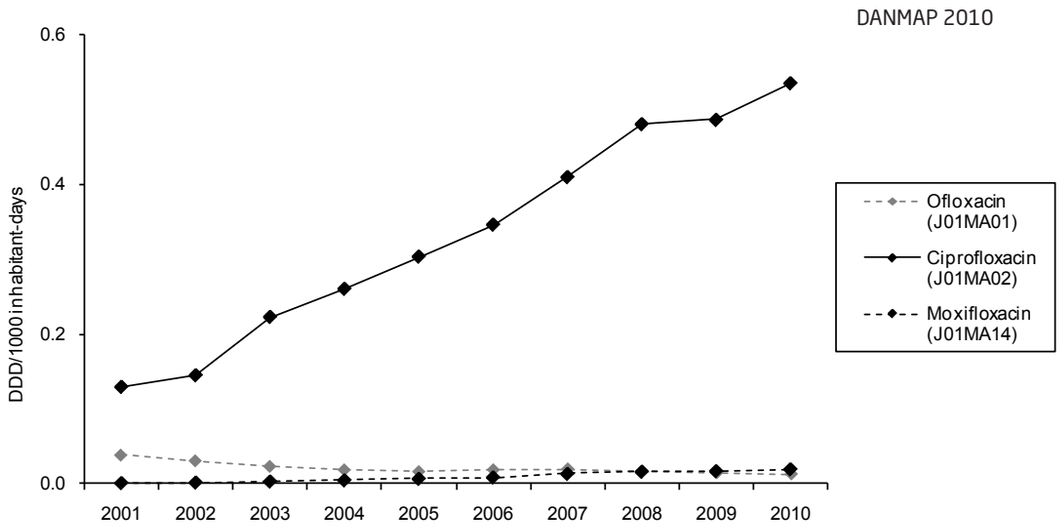
The trends in numbers of occupied bed-days and admissions to somatic hospitals 2001–2010 are shown in Figure 5.13. The regional numbers of occupied bed-days and admissions to somatic hospitals 2010 are displayed in table AP1.7 in appendix 1.

In Denmark, the hospitalization pattern has changed over the last decade. Today, more people are admitted to the somatic hospitals, but each length of stay has shortened. Also, outpatient treatment has increased. Consequently, the hospital activity and the selection pressure for the emergence of resistance are higher than 10 years ago.

In this report, the number of occupied bed-days and admissions of 2001–2009 obtained from the National Board of Health has been updated. This update has particularly affected the reported consumption of 2009 and resulted in only minor changes from 2001–2008.

Due to procedural rearrangements (merging of antibacterial agents and infusion liquids) of certain chemical substances for infusion, the reporting of sales (consumption) by the hospital pharmacies to the Danish Medicines Agency has been inaccurate for some groups. Consumption of cephalosporins, carbapenems, combinations of sulfonamides and trimethoprim and imidazole derivates has been corrected, as in previous years. In 2010, data were collected from all Danish hospital pharmacies.

Figure 5.12. Consumption of leading fluoroquinolones in primary health care, Denmark



5.4.2 Total consumption in hospital care - DDD per 1,000 inhabitants per day (DID)

Total consumption compared with 2009

Total consumption (J01) in Danish hospital care (rehabilitation centres, hospices, private-, psychiatric-, specialised-, and somatic hospitals) added up to 1.91 DID in 2010; similar to that of 2009 (Figure 5.14). Broad-spectrum agents represented 67% of the total consumption, as in 2009.

Total consumption - the last decade

Since 2001, total consumption has increased by 0.46 DID (31%). Broad-spectrum agents have increased by 0.18 DID (37%); comprising 67% of the total consumption in 2010 compared with 49% in 2001 (Figure 5.14 and Table AP 1.8 in appendix 1).

Figure 5.13. Number of bed-days and admissions in somatic hospitals, Denmark

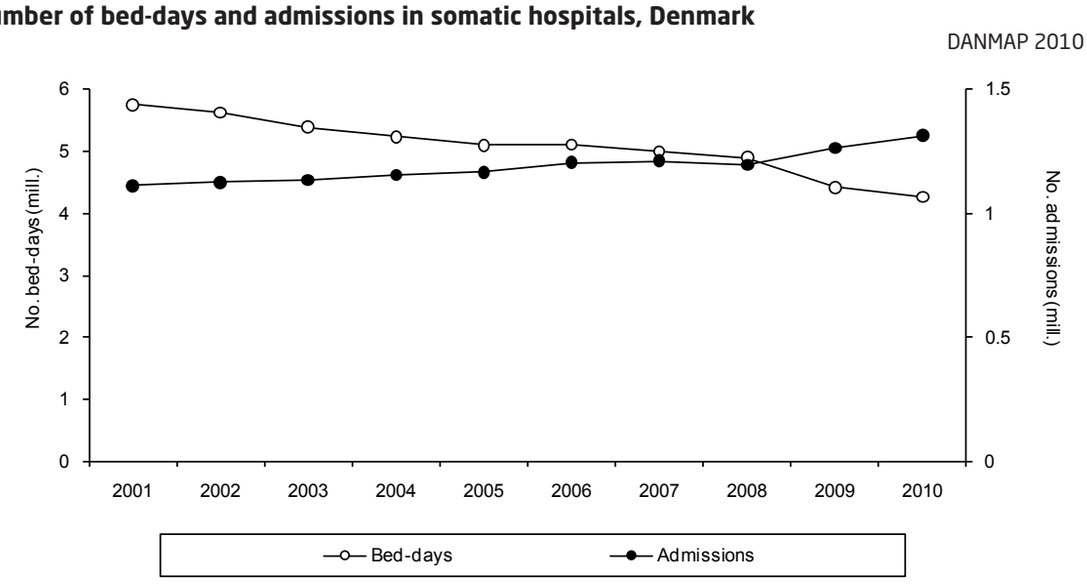
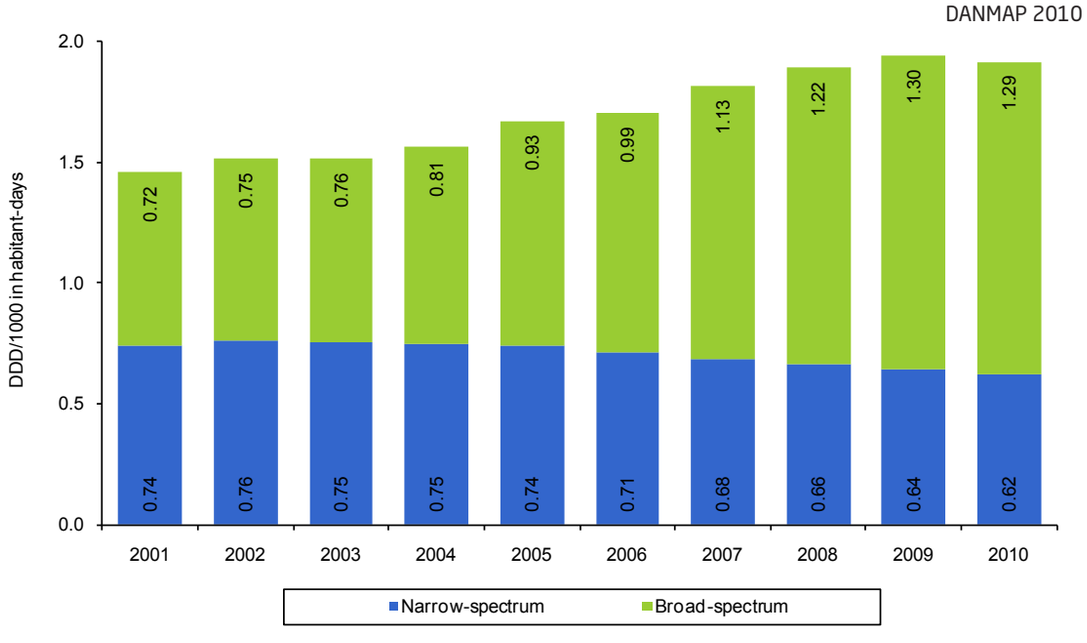


Figure 5.14. Consumption of antibacterial agents (J01) in hospital care by narrow-spectrum[(a)] and broad-spectrum[(b)] agents, Denmark



a) Narrow-spectrum antibiotics includes: beta-lactamase sensitive penicillins, first-generation cephalosporins, beta-lactamase resistant penicillins, monobactams, trimethoprim, sulfonamides, macrolides, lincosamides, glycopeptides, fusidic acid, imidazol derivatives, nitrofuran derivatives, and 'other antibiotics'.
b) Broad-spectrum includes: tetracyclines, penicillins with extended spectrum, combinations of penicillins incl. beta-lactamase inhibitors, second-generation cephalosporins, third-generation cephalosporins, carbapenems, combinations of sulfonamides and trimethoprim, aminoglycosides, fluoroquinolones, and polymyxins.

5.4.3 Somatic hospitals - DDD per 100 occupied bed-days (DBD)

Somatic hospital consumption (DBD) compared with 2009

Total consumption (J01) in somatic hospitals increased by 2.69 DBD (3%) from 2009 to 2010 (Table 5.5). Three therapeutic groups dominated the increase in consumption: 'combination penicillins' 1.48 DBD (26%), carbapenems 0.88 DBD (28%) and combinations of sulfonamides and trimethoprim 0.76 DBD (34%), but also second-generation cephalosporins, beta-lactamase resistant penicillins, macrolides and aminoglycosides increased ≥0.10 DBD. The increased consumption of the latter three was due to decreasing numbers of bed-days and practically equal numbers of DDDs. Regarding second-generation cephalosporins, consumption measured as DBD went up even though the number of DDDs used decreased compared with 2009.

An intervention at Copenhagen University Hospital, Bispebjerg changed the local consumption pattern, with lower consumption of cephalosporins and fluoroquinolones, but nationally it did not influence the consumption much [Textbox 8].

Consumption decreased ≥0.10 DBD in four therapeutic groups: penicillins with extended spectrum 0.76 DBD (5%), beta-lactamase sensitive penicillins 0.41 DBD (4%), third-generation cephalosporins 0.16 DBD (11%), and fluoroquinolones 0.27 DBD (2%). Regarding all four, the decreased consumption was due to both decreasing numbers of bed-days and DDDs.

Cephalosporins accounted for 20% of the total consumption in somatic hospitals. Penicillins with extended spectrum (17%), fluoroquinolones (12%) and beta-lactamase sensitive penicillins (11%) were the other top four contributing therapeutic groups in 2010 (Figure 5.15).

Somatic hospital consumption (DBD) - the last decade

During 2001–2010, the total consumption (J01) in somatic hospitals has increased by 39.40 DBD (82%) (Table 5.5). This increase was due to a 35% increase in the number of DDDs, and a concurrent 26% decrease in the total number of hospital bed-days.

Figure 5.15. Distribution of the total consumption of antibacterial agents in somatic hospitals, Denmark

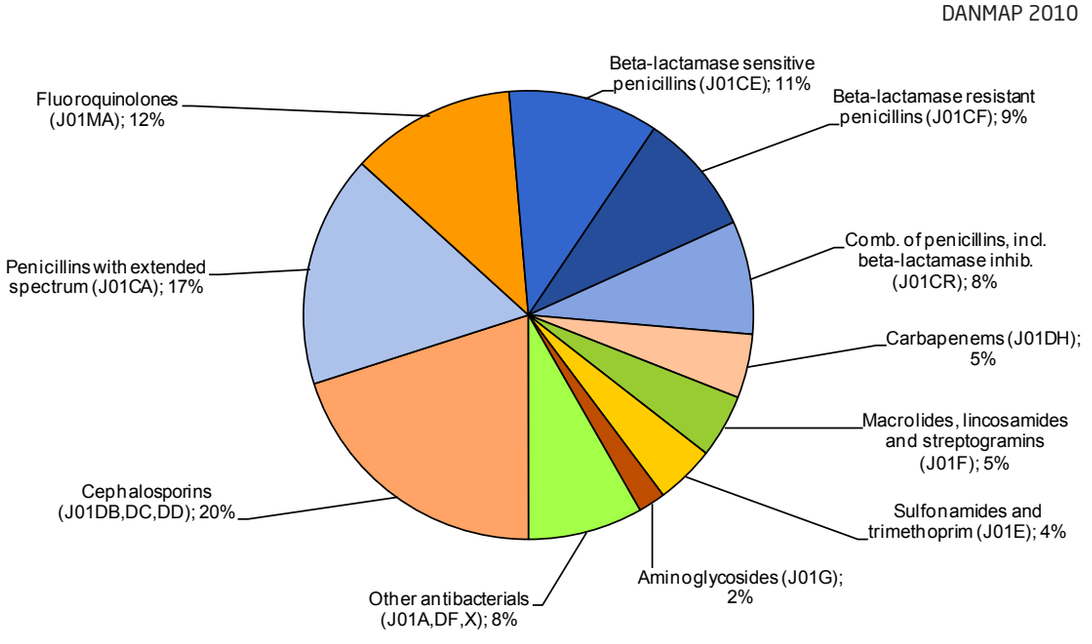


Table 5.5. Consumption of antibacterial agents for systemic use in somatic hospitals (DDD/100 occupied bed-days), Denmark

DANMAP 2010

ATC group[a]	Therapeutic group	Year									
		2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
J01AA	Tetracyclines	0.28	0.32	0.30	0.32	0.33	0.39	0.63	0.78	1.04	1.09
J01CA	Penicillins with extended spectrum	11.33	11.34	11.55	11.51	12.90	13.00	13.42	13.96	15.37	14.61
J01CE	Beta-lactamase sensitive penicillins	10.45	11.37	11.85	12.02	12.17	10.67	10.79	9.98	9.90	9.49
J01CF	Beta-lactamase resistant penicillins	5.90	6.24	6.54	6.78	6.71	6.51	6.70	6.81	7.40	7.71
J01CR	Combinations of penicillins. incl. beta-lactamase inhibitors	0.17	0.31	0.49	0.84	1.16	1.83	2.95	4.00	5.65	7.13
J01DB	First-generation cephalosporins	0.11	0.14	0.14	0.17	0.15	0.14	0.13	0.18	0.13	0.13
J01DC	Second-generation cephalosporins	5.12	5.79	6.24	6.91	8.39	9.38	12.31	13.32	15.76	16.21
J01DD	Third-generation cephalosporins	0.65	0.65	0.67	0.67	0.83	0.83	1.03	1.25	1.42	1.26
J01DF	Monobactams	0.01	0.00	0.01	0.00	0.00	0.00	0.04	0.07	0.06	0.09
J01DH	Carbapenems	0.42	0.60	0.68	0.85	1.16	1.38	2.13	2.70	3.15	4.02
J01EA	Trimethoprim and derivatives	0.42	0.41	0.43	0.41	0.41	0.42	0.44	0.44	0.44	0.36
J01EB	Short-acting sulfonamides	1.22	1.23	1.14	1.06	0.99	0.75	0.34	0.35	0.35	0.33
J01EE	Combinations of sulfonamides and trimethoprim. incl. derivatives	1.33	1.46	1.54	1.86	2.11	2.12	1.52	1.95	2.28	3.04
J01FA	Macrolides	3.21	3.21	2.95	2.85	2.89	2.83	3.08	3.06	3.42	3.52
J01FF	Lincosamides	0.17	0.19	0.22	0.23	0.24	0.31	0.35	0.41	0.50	0.47
J01GB	Aminoglycosides	1.82	1.76	1.71	2.00	1.95	1.81	1.79	1.64	1.56	1.71
J01MA	Fluoroquinolones	2.81	3.52	3.90	4.93	6.14	6.74	8.16	9.53	10.71	10.44
J01XA	Glycopeptides	0.32	0.38	0.42	0.47	0.52	0.56	0.63	0.68	0.99	1.07
J01XB	Polymyxins	0.03	0.04	0.03	0.06	0.12	0.12	0.05	0.05	0.07	0.10
J01XC	Steroid antibacterials (fusidic acid)	0.19	0.19	0.22	0.22	0.25	0.28	0.28	0.26	0.31	0.34
J01XD	Imidazole derivatives	1.94	2.12	2.32	2.43	2.62	2.78	2.62	3.27	3.84	3.93
J01XE	Nitrofuran derivatives (nitrofurantoin)	0.28	0.28	0.30	0.28	0.29	0.29	0.28	0.29	0.36	0.31
J01XX05	Methenamine	0.13	0.12	0.08	0.10	0.08	0.11	0.09	0.10	0.09	0.08
J01XX08	Linezolid	0.00	0.04	0.04	0.07	0.15	0.20	0.16	0.21	0.22	0.22
J01XX09	Daptomycin	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.02	0.02	0.02
J01	Antibacterial agents for systemic use (total)	48.31	51.73	53.77	57.04	62.58	63.47	69.94	75.28	85.03	87.72

a) From the 2010 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Figure 5.16 illustrates the steady shift towards increasing consumption of 'newer' broad-spectrum antibacterial agents - defined as: 'combination penicillins' (J01CR), cephalosporins (J01DB, DC, DD), carbapenems (J01DH) and fluoroquinolones (J01MA) - in Danish somatic hospitals. In 2001, consumption of penicillins with extended spectrum and beta-lactamase sensitive penicillins represented 24% and 22% of total somatic hospital antibacte-rial consumption in Denmark, respectively. These shares had decreased to 17% and 11% in 2010. Within the group of the penicillins with extended spectrum, the decrease mainly concerned ampicillin/pivampicillin/amoxicillin whereas consumption of mecillinam/pivmecillinam has increased. Consumption of cephalosporins represented 12% of total somatic hospital antibacterial consumption in 2001, rising to 20% in 2010.

The benefit of these changes in patterns of antibacterial consumption over the last decade could be an empirical treatment covering more groups of pathogens. Nevertheless, this potential gain seems to be rapidly counterbalanced by the emergence of resistance towards newer classes of antibacterial agents (see chapter 8). Importantly, it is interesting that the steeply increasing proportion of cephalosporins and fluoroquinolones used during the last decade has yielded over the last two years.

5.4.4 Somatic hospital consumption - DDD per 100 admissions (DAD)

Somatic hospital consumption (DAD) compared with 2009

The total consumption (J01) in somatic hospitals decreased by 12.47 (4%) from 2009–2010 when expressed as the number of DAD (Table 5.6). As when measured as DBD, consumption increased in the same three therapeutic groups by ≥1 DAD: 'combination penicillins' 3.41 DAD (17%), carbapenems 2.06 DAD (28%) and combinations of sulfonamides and trimethoprim 1.91 DAD (24%). Consumption decreased ≥1 DAD in four therapeutic groups: penicillins with extended spectrum 6.31 DAD (12%), beta-lactamase sensitive penicillins 3.78 DAD (11%), second-generation cephalosporins 2.47 DAD (4%), and fluoroquinolones 3.53 DAD (9%).

Somatic hospital consumption (DAD) - the last decade

Antibacterial consumption (J01) has increased by 14% from 249.76 DAD in 2001 to 284.89 DAD in 2010 (Table 5.6). This increase was driven by a 35% increase in the number of DDDs, but counterbalanced by an 18% increase in the number of admissions during the last decade (as a consequence of changes in hospitalization patterns).

The difference between trends in consumption measured by DBD and DAD illustrates that the interpretation of the measures of consumption is highly dependent on the denominator as well as the nominator (DDD) and that one indicator is not enough to express hospital consumption.

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Figure 5.16. Percentages of total somatic hospital consumption by leading groups of antibacterial agents (J01), Denmark

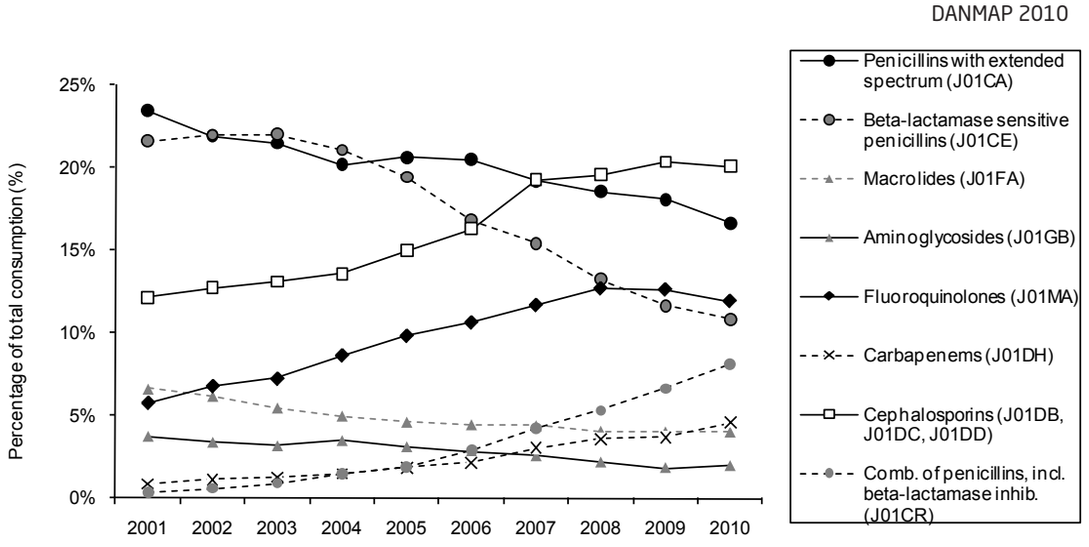


Table 5.6. Consumption of antibacterial agents for systemic use in somatic hospitals (DDD/100 admitted patients), Denmark

DANMAP 2010

ATC group[a]	Therapeutic group	Year									
		2001	2002	2003	2004	2005	2006	2007	**2008[b]**	2009	2010
J01AA	Tetracyclines	1.44	1.58	1.43	1.45	1.45	1.67	2.59	**3.19**	3.63	3.55
J01CA	Penicillins with extended spectrum	58.59	56.68	54.88	52.22	56.43	55.13	55.39	**57.18**	53.76	47.46
J01CE	Beta-lactamase sensitive penicillins	54.03	56.79	56.33	54.53	53.20	45.26	44.55	**40.90**	34.61	30.83
J01CF	Beta-lactamase resistant penicillins	30.50	31.18	31.11	30.77	29.33	27.60	27.64	**27.89**	25.86	25.04
J01CR	Comb. of penicillins. incl. beta-lactamase inhibitors	0.88	1.56	2.35	3.82	5.09	7.77	12.17	**16.37**	19.74	23.15
J01DB	First-generation cephalosporins	0.58	0.72	0.67	0.76	0.67	0.60	0.55	**0.72**	0.46	0.43
J01DC	Second-generation cephalosporins	26.48	28.95	29.66	31.36	36.70	39.76	50.81	**54.55**	55.12	52.65
J01DD	Third-generation cephalosporins	3.34	3.25	3.17	3.06	3.62	3.53	4.24	**5.10**	4.98	4.10
J01DF	Monobactams	0.05	0.02	0.02	0.02	0.02	0.00	0.18	**0.27**	0.21	0.29
J01DH	Carbapenems	2.16	2.98	3.24	3.85	5.05	5.86	8.78	**11.08**	11.01	13.07
J01EA	Trimethoprim and derivatives	2.19	2.07	2.05	1.86	1.78	1.78	1.81	**1.80**	1.56	1.17
J01EB	Short-acting sulfonamides	6.33	6.16	5.44	4.82	4.32	3.18	1.41	**1.43**	1.21	1.09
J01EE	Comb. of sulfonamides and trimethoprim. incl. derivatives	6.88	7.31	7.32	8.44	9.21	8.98	6.28	**7.98**	7.96	9.88
J01FA	Macrolides	16.58	16.03	14.03	12.92	12.64	12.01	12.70	**12.53**	11.97	11.45
J01FF	Lincosamides	0.88	0.94	1.05	1.04	1.05	1.31	1.46	**1.69**	1.74	1.52
J01GB	Aminoglycosides	9.42	8.79	8.14	9.07	8.55	7.68	7.39	**6.71**	5.45	5.56
J01MA	Fluoroquinolones	14.51	17.60	18.53	22.38	26.87	28.58	33.66	**39.04**	37.45	33.92
J01XA	Glycopeptides	1.63	1.88	1.97	2.12	2.28	2.38	2.61	**2.77**	3.48	3.47
J01XB	Polymyxins	0.15	0.20	0.14	0.27	0.54	0.53	0.22	**0.21**	0.24	0.32
J01XC	Steroid antibacterials (fusidic acid)	1.01	0.97	1.04	1.01	1.11	1.19	1.17	**1.05**	1.09	1.12
J01XD	Imidazole derivatives	10.02	10.57	11.03	11.02	11.47	11.81	10.83	**13.39**	13.43	12.76
J01XE	Nitrofuran derivatives (nitrofurantoin)	1.47	1.42	1.41	1.26	1.28	1.24	1.17	**1.19**	1.27	1.01
J01XX05	Methenamine	0.65	0.61	0.37	0.45	0.36	0.46	0.38	**0.43**	0.31	0.27
J01XX08	Linezolid	0.00	0.22	0.21	0.34	0.64	0.86	0.68	**0.84**	0.76	0.72
J01XX09	Daptomycin	0.00	0.00	0.00	0.00	0.00	0.00	0.03	**0.06**	0.06	0.07
J01	Antibacterial agents for systemic use (total)	249.76	258.46	255.59	258.81	273.67	269.18	288.70	**308.39**	297.36	284.89

a) From the 2010 edition of the Anatomical Therapeutic Chemical (ATC) classification system
b) The number of admissions was affectedly low in 2008 due to a major hospital strike

6 RESISTANCE IN ZOONOTIC BACTERIA

6. Resistance in zoonotic bacteria

Zoonoses are infections and diseases that are transmissible between animals and humans, either via direct contact or indirectly via contaminated food. Zoonotic bacteria such as *Salmonella* and *Campylobacter* can develop resistance to antimicrobial agents as a result of treatment of the animals, which subsequently may lead to treatment failure of human infections. A more detailed description of the trends and sources of zoonoses in Denmark and of national surveillance and control programmes can be found in the Annual Report on Zoonoses in Denmark 2010 [www.food.dtu.dk].

6.1 *Salmonella*

Salmonella is an important zoonotic pathogen with great economic significance in both animals and humans. The common reservoir of *Salmonella* is the intestinal tract of a wide range of domestic and wild animals. In animals, infections are often sub-clinical. Transmission of *Salmonella* to humans often happens through a food vehicle which has been contaminated with *Salmonella* and where the organisms have been allowed to multiply due to e.g. inadequate storage temperatures, inadequate cooking or cross contamination of ready-to-eat food. In Denmark, as well as in the European Union, *S.* Enteritidis and *S.* Typhimurium are the serovars most frequently associated with human illness. Human cases caused by *S.* Enteritidis are mostly associated with the consumption of contaminated eggs and poultry meat, while *S.* Typhimurium cases are mostly associated with the consumption of contaminated pig, poultry and bovine meat.

Human salmonellosis is typically characterized by the acute onset of fever, abdominal pain, nausea, and sometimes vomiting, after an incubation period of 12–36 hours. Symptoms are often mild and most infections are self-limiting. However, in some patients the infection may be more serious, and salmonellosis has also been associated with long-term and chronic sequelae such as reactive arthritis.

In Denmark, all flocks of laying hens and broilers, including breeder flocks, are monitored for *Salmonella* according to the EU requirements. Eggs from *Salmonella* positive laying hen flocks are heat treated or destroyed, and meat from broiler flocks found positive at the ante-mortem control is heat treated. An extensive *Salmonella* surveillance and control program is also running in the Danish pig production (at herd level) and samples of pork and beef are collected after chilling at the slaughterhouses. Finally, a control program for *Salmonella* in Danish and imported broiler meat, beef and pork has been implemented. Human salmonellosis is notifiable in Denmark, and all cases are reported to the national database at SSI.

Clonal dissemination plays an important role for the spread of antimicrobial resistant *Salmonella* spp., particularly within *S.* Typhimurium. Examples of this are the rapid, global dissemination of the penta-resistant *S.* Typhimurium DT104, which is resistant to ampicillin (A), chloramphenicol (C), streptomycin (S), sulfonamide (Su) and tetracycline (T) (ACSSuT), and the emergence of the monophasic "*S.* Typhimurium-like" strains. In addition, and presumably as a consequence of clonal dissemination, there also appears to be strong associations between certain phage types and particular resistance patterns. Again *S.* Typhimurium DT104 is an example of this, and so are the monophasic *S.* Typhimurium DT193 and DT120 that are typically resistant to ampicillin (A), streptomycin (S), sulfonamide (Su) and tetracycline (T), known as the 'classic DT120 pattern' or ASSuT. However, DT193 and DT120 are also commonly found in other lineages, for instance lineages derived from DT104, where they may show other resistance patterns such as ACSSuT.

Some phage types (e.g. DT12 and DT66) appear to only very slowly acquire resistance - if at all. Rather, such phage types appear to be displaced by resistant phage types if a selection pressure is put on them due to changes in the use of antimicrobial agents.

In this report, monophasic *Salmonella* isolates are included as *S.* Typhimurium, as recently recommended by the European Food Safety Authority [EFSA journal 2010. 8(10): 1826]. For animals and meat, the data from 2005 to 2009 have been updated accordingly. See definition of multi-resistance in Appendix 2.

6.2 *Salmonella* from production animals

For pigs and poultry, the isolates originated mainly from national surveillance programs. All Danish broiler flocks and table egg layer flocks were tested for *Salmonella* during 2010 (3,773 and 455 flocks, respectively), of which 43 broiler flocks and eight layer flocks were positive. Overall, nine flocks were found positive with *S.* Typhimurium and five flocks with *S.* Enteritidis [Annual Report on Zoonoses in Denmark 2010]. *S.* Typhimurium was isolated from 434 of the 1,089 pig herds appointed for testing based on results from the sero-surveillance. In addition, 21 isolates from diagnostic submissions were included. All 18 isolates from cattle were from clinical submissions.

Among the isolates tested for antimicrobial resistance in 2010, one isolate per farm was randomly included in the report. Insufficient numbers of *S.* Typhimurium and *S.* Enteritidis isolates (≥15) were obtained for Danish broilers and layers, and the results of the susceptibility testing is not presented.

MIC distributions among *S.* Typhimurium from cattle and pigs in 2010 are shown in Appendix 1 (Table AP1.9).

In 2010, none of the isolates of *S.* Typhimurium or *S.* Enteritidis from pigs or cattle were found resistant to cephalosporins, ciprofloxacin or nalidixic acid.

Salmonella Typhimurium in cattle

Of the 18 *S.* Typhimurium isolates that were susceptibility tested, 22% were fully sensitive and 56% were found to be multi-resistant. The highest occurrence of resistance was to streptomycin (67%), followed by tetracycline (61%), sulfonamide (56%) and ampicillin (56%) (Table 6.1). Nine of the isolates had the ASSuT resistance pattern, primarily phage type DT193 (6 isolates), but also DT120, DT7 and DTU302.

Salmonella Typhimurium in pigs

Among the selected 455 *S.* Typhimurium isolates from pigs, the most common phage types were DT120 (23%), DT193 (19%), DT12 (9%) and DT104 (7%). Overall, 38% of the susceptibility tested *S.* Typhimurium isolates from pigs were fully sensitive, whereas 53% were found to be multi-resistant. The highest occurrence of resistance was to streptomycin (56%), followed by sulfonamide (53%), ampicillin (49%) and tetracycline (47%) (Table 6.1).

Since 2000, there has been a parallel increase in resistance to ampicillin, sulfonamide and tetracycline (Figure 6.1). In 2008 and 2009, a temporary reduction in tetracycline resistance was observed which has not been fully explained; although it was partly related to a reduction in DT104 and other phage types often resistant to tetracycline. However, in 2010 the occurrence of resistance to tetracycline increased again (Figure 6.1), which is explained in part by an increase in the monophasic DT193 with the resistance pattern ASSuT (Figure 6.2). In addition, the significant increase in resistance to tetracycline coincided with an 5% decrease in consumption of tetracyclines per pig produced supporting that the increase is better explained by the spread of resistant clones.

The level of resistance in 2010 was significantly higher for ampicillin, streptomycin and tetracycline, as compared to the 2009 levels.

The proportion of *S.* Typhimurium isolates with the ASSuT resistance pattern increased from 15% to 29% from 2005 to 2010. The majority of the isolates with the ASSuT resistance pattern was DT120, and to some extent the monophasic DT193 which increased markedly from 2009 to 2010. The increased occurrence of the ASSuT resistance pattern in *S.* Typhimurium isolates is primarily a reflection of the increased occurrence of these phage types in the Danish pig population (Figure 6.2).

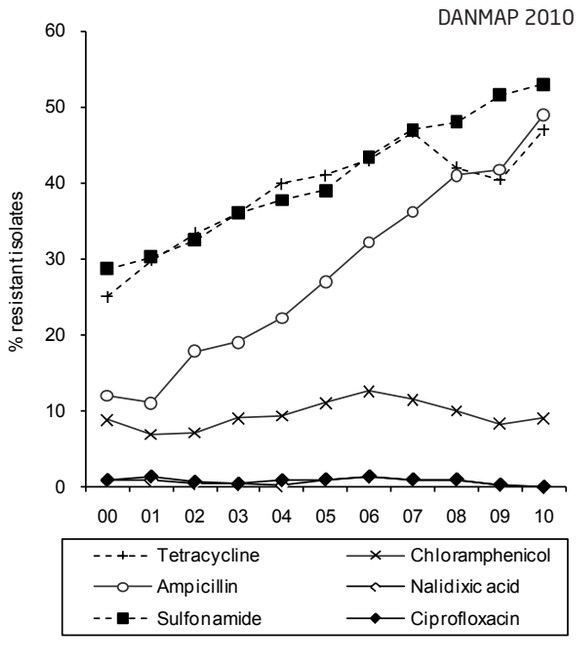
Table 6.1. Resistance (%) among *Salmonella* Typhimurium from cattle, pigs, different types of meat and domestic sporadic human cases, Denmark

DANMAP 2010

Antimicrobial agent	Cattle	Pigs	Pork		Broiler meat	Turkey meat	Human
	%	%	Danish %	Imported %	Imported %	Imported %	Domestic sporadic[(a)] %
Tetracycline	61	47	27	77	6	100	36
Chloramphenicol	6	9	4	21	0	10	7
Florfenicol	6	6	4	10	0	10	6
Ampicillin	56	49	35	73	11	68	42
Ceftiofur	0	0	0	0	0	2	0
Cefotaxime	0	0	0	0	0	2	-
Sulfonamide	56	53	38	84	11	93	44
Trimethoprim	0	8	0	18	0	27	5
Apramycin	0	1	0	0	0	24	1
Gentamicin	0	2	0	0	0	27	1
Neomycin	0	3	0	3	0	24	4
Spectinomycin	6	16	8	24	0	39	9
Streptomycin	67	56	46	87	28	93	43
Ciprofloxacin	0	0	0	0	0	2	4
Nalidixic acid	0	0	0	0	0	2	2
Colistin	0	0	0	0	0	2	1
Number of isolates	18	455	26	62	18	41	227

a) The isolate was categorized as 'domestic sporadic' if the patient did not travel one week prior to the infection and was not reported as beeing part of an outbreak

Figure 6.1. Resistance (%) in *Salmonella* Typhimurium[a] from pigs, Denmark



a) The number of isolates varies between years (from 216 to 736)

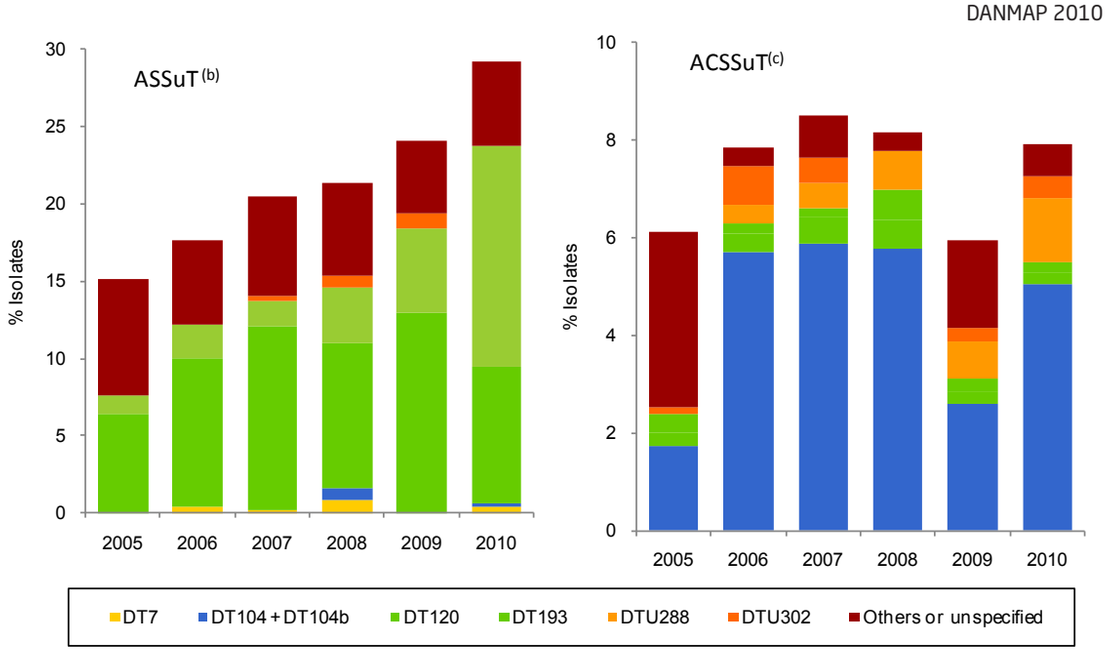
The proportion of *S.* Typhimurium isolates with the ACSSuT resistance pattern varied between 6% and 8% during the period 2005–2009, and was 8% in 2010. The majority of the isolates with the ACSSuT resistance pattern was DT104 or DT104b, and the changes in occurrence of the ACSSuT resistance pattern were mainly due to changes in the occurrence of phage type DT104, although an increase in ACSSuT resistant DTU288 appears to have occurred over the last years (Figure 6.2).

6.2.1 *Salmonella* in meat

Salmonella isolates from Danish and imported broiler meat, turkey meat, beef and pork are collected as part of the national case-by-case control programme. *Salmonella* was not detected in any of the 97 batches from Danish broiler meat tested in 2010, whereas it was isolated from 12% (58/490) of the batches of imported broiler meat. *Salmonella* was detected in 9% (56/592) of the batches of imported turkey meat tested in 2010, with several isolates per positive batch. In imported beef and pork, 3% (4/127) and 14% (40/296) of the batches tested *Salmonella* positive, respectively [Annual Report on Zoonoses in Denmark 2010].

Salmonella isolates from Danish pork and beef originate from the National surveillance programme, where carcass swab samples are collected at the slaughterhouses. In Danish pork, 22,485 pooled samples (each of five carcasses) were analysed in 2010, and an estimated 1.2% of the pig carcasses

Figure 6.2. Proportional distibution of phage types among *Salmonella* Typimurium isolates[a] from pigs resistant to ampicillin, streptomycin, sulfonamide and tetracycline without or with chloramphenicol resistance (ASSuT/ACSSuT), Denmark



a) Total number of isolates included in 2005: n = 752; 2006: n = 509; 2007: n = 577; 2008: n = 502; 2009: n = 386 and 2010: n = 455
b) Isolates with 'ASSuT' can also include resistence to other antimicrobial agents except chloramphenicol
c) Isolates with 'ACSSuT' all include resistance to chloramphenicol but can also include resistance to other antimicrobial agents

were *Salmonella* positive. In addition, 223 individual carcasses were tested in smaller slaughterhouses, where 1.8% of the pig samples were *Salmonella* positive. In Danish beef, 7,660 pooled samples (each of five carcasses) were analysed in 2010, and an estimated 0.3% of the cattle carcasses were *Salmonella* positive. In addition, 162 individual carcasses were tested in the smaller slaughterhouses, with no *Salmonella* positive [Annual Report on Zoonoses in Denmark 2010].

All susceptibility tested *S.* Typhimurium and *S.* Enteritidis isolates were included, even in those cases where more than one isolate was found per tested batch or pooled sample. Insufficient numbers of *S.* Typhimurium isolates (≥15) were obtained for Danish broiler meat, Danish beef and imported beef, and the results of the susceptibility testing is not presented in this report. Due to a low number of isolates, resistance data from *S.* Enteritidis in meat are also excluded.

MIC distributions among *S.* Typhimurium from imported broiler meat, imported turkey meat and pork (Danish and imported) in 2010 are shown in Appendix 1 (Table AP1.10).

In 2010, only *S.* Typhimurium isolates from imported turkey meat were found resistant to cephalosporins (2%), ciprofloxacin (2%) and nalidixic acid (2%), whereas all *S.* Typhimurium isolates from imported broiler meat and pork were sensitive towards these three antimicrobial agents (Table 6.1).

Salmonella Typhimurium in imported broiler meat

There were 18 *S.* Typhimurium isolates among the 137 susceptibility tested *Salmonella* isolates selected from imported broiler meat, where the most frequent phage type was DTU312 (83%). Overall, 13 (72%) of the susceptibility tested *S.* Typhimurium isolates from imported broiler meat were fully sensitive, whereas two (11%) were multi-resistant. One isolate (6%) was resistant to tetracycline, two isolates (11%) to ampicillin and sulfonamide, and five isolates (28%) were resistant to streptomycin (Table 6.1). None of the isolates were resistant to ciprofloxacin and nalidixic acid.

Salmonella Typhimurium in turkey meat

The vast majority of Danish turkeys are exported for slaughter; therefore, no *Salmonella* isolates were available for susceptibility testing from Danish turkey meat in 2010.

Among the 163 susceptibility tested *Salmonella* isolates selected from imported turkey meat, 41 *S.* Typhimurium isolates were found; where the monophasic Typhimurium strain 4,5,12:i:- accounted for almost half. The most common phage type was DT193 (59%), but DT104/104b was also reported. The highest levels of resistance was found in the imported turkey meat, where none of the susceptibility tested *S.* Typhimurium isolates were fully sensitive, and 93% were found to be multi-resistant. In one *S.* Typhimurium isolates from imported turkey meat, resistance was found to all tested antimicrobial agents (Table 6.1). All isolates were tetracycline resistant, and the occurrence of resistance to sulfonamide (93%), streptomycin (93%) and ampicillin (68%) was also very high.

Furthermore, imported turkey meat was the only food source where resistant to ceftiofur and cefotaxime was found. In 2010, a significant increase in *S.* Typhimurium isolated from imported turkey meat was seen resistant to apramycin (from 0% to 24%), gentamicin (from 0% to 27%) and streptomycin (from 69% to 93%) compared with 2009.

Salmonella Typhimurium in pork

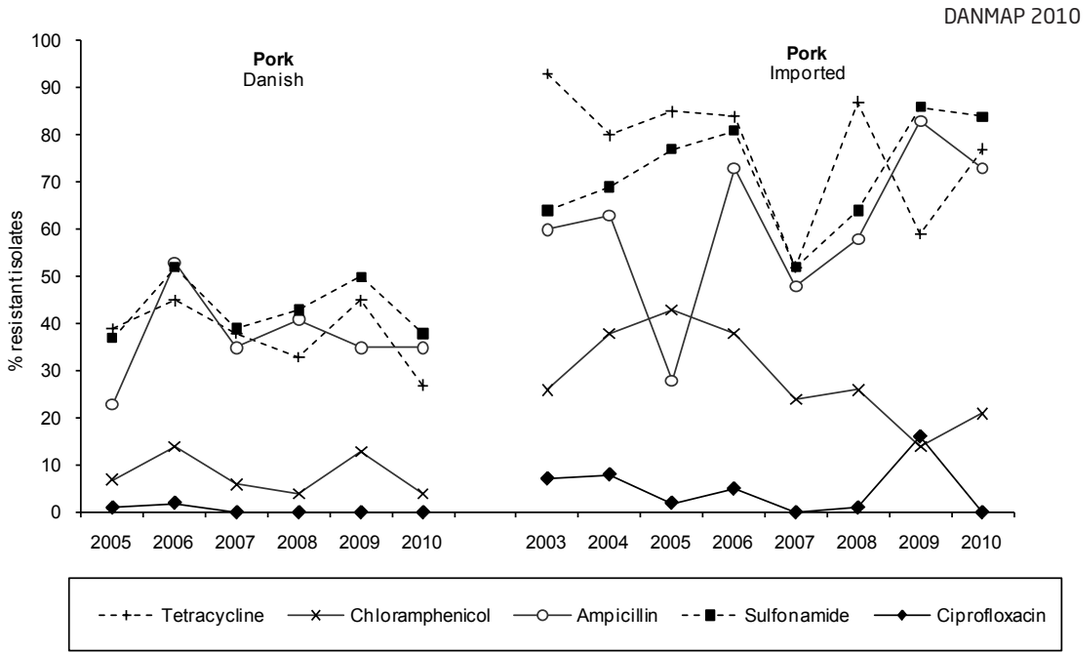
Among the 26 *S.* Typhimurium isolates from Danish pork that were susceptibility tested, the most dominant phage types were DT120 (31%) and DT12 (23%), followed by DT17 (8%), DT7(8%), DTU66 (4%), DT193 (4%) and DTU302 (4%). Overall, 46% of the susceptibility tested *S.* Typhimurium isolates were fully sensitive, whereas 35% were found to be multi-resistant.

Resistance to ampicillin (35%), streptomycin (46%), sulfonamide (38%) and tetracycline (27%) was most common (Table 6.1). There was no significant change in the level of resistance in the susceptibility tested *S.* Typhimurium isolates from Danish pork in 2010 compared to 2009. When comparing the resistance in Danish pork to resistance in Danish pigs, a significantly higher occurrence of resistance to tetracycline (27% vs. 47%) was found in the animals (Table 6.1).

In imported pork, 99 susceptibility tested *Salmonella* isolates were selected, and among the 62 *S.* Typhimurium isolates the most common phage types were DT120 (45%) and DT17 (32%). Overall, 10% of the *S.* Typhimurium isolates were fully sensitive, whereas 84% were found to be multi-resistant. The highest levels of resistance were to streptomycin (87%), sulfonamide (84%), tetracycline (77%) and ampicillin (73%) (Table 6.1). In the isolates from imported pork, none of the isolates were resistant to nalidixic acid and ciprofloxacin, a significant decrease compared with 2009 (16%). At the same time, a significant increase in resistance to streptomycin (42%) and trimethoprim (344%, from 5% to 18%) was observed.

S. Typhimurium isolates from imported pork had a significant higher occurrence of resistance to ampicillin, chloramphinicol, florfenicol, spectinomycin, streptomycin, sulfonamide, tetracycline and trimethoprim than *S.* Typhimurium isolates from Danish pork (Figure 6.3).

Figure 6.3. Resistance (%) in *Salmonella* Typhimurium[a] isolated from Danish and imported pork, Denmark



a) The number of isolates varies between years: for Danish pork from 64 to 103, and imported pork from 21 to 137. For Danish pork, data from before 2005 are not shown due to a low number of isolates

6.2.2 *Salmonella* in humans

In 2010, the number of human cases of salmonellosis decreased to 1,598 cases (29 per 100,000 inhabitants), compared with 2,129 cases reported in 2009. Of the 1,598 cases, information on susceptibility to antimicrobial agents was available for 1,508 isolates; of these, 629 isolates were *S.* Typhimurium and 364 isolates were *S.* Enteritidis. The remaining 515 isolates belonged to 97 other serovars.

MIC distributions among *S.* Typhimurium and *S.* Enteritidis from human cases in 2010 are shown in Appendix 1 (Tables AP1.11 and AP1.12).

SSI collected travel information from the patients diagnosed with salmonellosis by phone interviews. The patients were asked about the date of disease onset and whether they had travelled abroad within a seven-day period prior to disease. Patients who had travelled were asked about their destinations. Cases were categorized as "domestically acquired" if the patient had not travelled during that time period, and categorized as "travel abroad reported" if the patient had travelled. Cases with no travel information reported to the general practitioners, and where no phone interview was conducted, were categorized as "unknown origin". In 2010, travel information was obtained for 80% of the *Salmonella* cases.

Outbreaks of human salmonellosis are reported in the "Annual Report on Zoonoses in Denmark in 2010". All human cases associated with a detected outbreak were considered outbreak-related in this report.

Salmonella Typhimurium in humans

Fifteen percent of human *S.* Typhimurium cases were categorized as travel-related and 70% as domestically acquired. Of the domestically acquired, 48% were found to be part of an outbreak and 52% were domestic sporadic cases. Travel information was not available from 15% of the cases (Table 6.2).

Of the 217 cases of salmonellosis that were part of domestic outbreaks, 90% were caused by *S.* Typhimurium and 1% by *S.* Enteritidis. Among the 212 domestic outbreak-related human isolates of *S.* Typhimurium, 78% were phage type DTU323, and the remaining cases were caused by phage types DT120, DT5, DT7, DTU311, DT104 and DT193. Overall, 8% of the human *S.* Typhimurium isolates from domestic outbreaks were fully sensitive whereas 78% were multi-resistant.

The most commonly reported phage types in domestic sporadic human *S.* Typhimurium isolates were DT193 (20%), DT120 (15%) and DTU292 (11%). Overall, 49% of the sporadic domestic human *S.* Typhimurium isolates were fully sensitive whereas 43% were multi-resistant. For the travel-related cases and cases of unknown origin, 32% and 31% of the *S.* Typhimurium isolates were fully sensitive, whereas 63% and 60% were multi-resistant, respectively.

Among the human *S.* Typhimurium isolates, 21% of the domestic sporadic cases and 41% of the travel-related cases had the ASSuT resistance pattern. Among the ASSuT isolates, DT193 and DT120 were the most common phage types, but DT7 also represented a substantial part of the ASSuT isolates associated with the domestic outbreaks (Figure 6.5).

The ACSSuT resistance pattern was observed in 7% of the domestic sporadic cases and in 12% of the travel-related cases. The dominant phage type was DT104, but among the travel associated cases DT120 and DT193 were also common (Figure 6.5).

In the human *S.* Typhimurium isolates, the pattern of resistance was similar to what is currently observed in the rest of Europe, with resistance to ampicillin, streptomycin, sulfonamide and tetracycline dominating. The occurrence of resistance to these four antimicrobial agents was similar among travel-related cases and cases with unknown origin. In the domestic cases, the occurrence of resistance in the sporadic cases was markedly lower for all four antimicrobial agents than what was observed in the travel-related cases and the cases of unknown origin (Figure 6.4 and Figure 6.5). In the domestic outbreak-related cases, tetracycline resistance was much lower than what was observed in any other source of origin, but resistance to ampicillin, streptomycin and sulfonamide was much higher (Table 6.2).

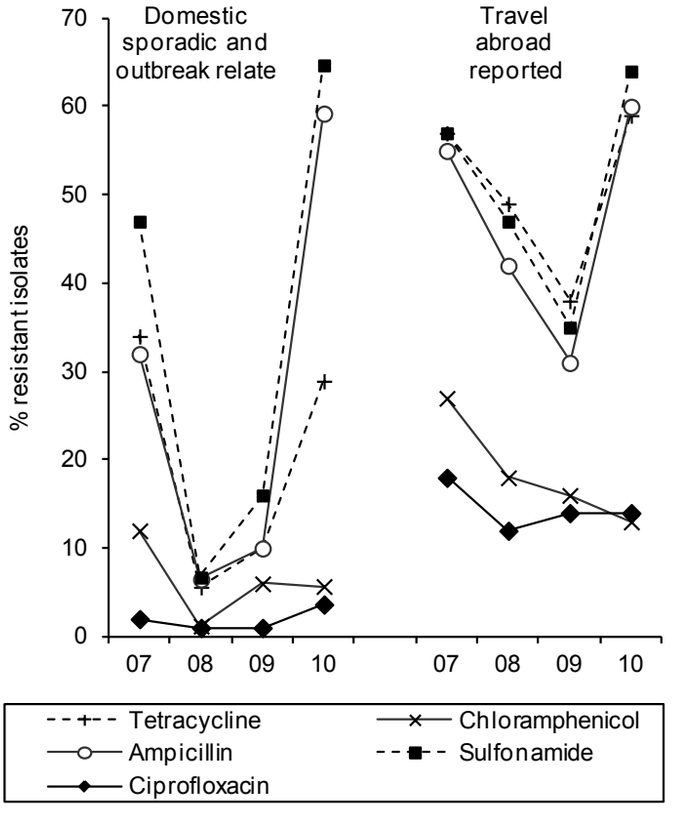
Resistance to cefalosporins was only found among the isolates from travel-associated cases (3%) or from cases where the source of infection was unknown (1%).

The higher level of ciprofloxacin resistance in the travel-associated *S.* Typhimurium infections (14%) when compared to the domestically acquired infections (4%) may reflect a higher consumption of fluoroquinolones in production animals in the countries of destination.

The occurrence of resistance to ciprofloxacin was higher than to nalidixic acid among human isolates due to the occurrence of the plasmid-borne *qnrS* genes which confer resistance to ciprofloxacin only. Of the ten human isolates resistant to ciprofloxacin but sensitive to nalidixic acid, nine had *qnr* genes. The four ceftiofur resistant isolates, three from travel associated infections and one from an unknown infection, were all producing the ESBL-enzymes belonging to the CTX-M-1-group.

Figure 6.4. Resistance (%) in *Salmonella* Typhimurium[a] in human cases acquired[b] domestically or associated with travel, Denmark

DANMAP 2010



Note: that the shape of the curves are highly influenced by several large outbreaks with fully sensitive strains in 2008 and 2009 and a large outbreak with a strain resistant to Ampicillin, Streptomycin and sulfonamide in 2010. Verified monophasic S. Typhimurium-like isolates are included in 2010.

a) Number of isolates included as domestic and travel related: 2007 = 90 and 44, 2008 = 1,441 and 103, 2009 = 560 and 58, 2010 = 439 and 95

b) The isolate was categorized as 'domestically acquired' if the patient did not travel one week prior to the infection, and it was characterized as 'travel abroad reported' if the patient travelled one week prior to the infection

ERRATUM: In the printed version of DANMAP 2010 and in digital versions from before 15. Nov 2011, Figure 6.4 include 2007-2009 data from all domestic *Salmonella* cases (sporadic and outbreak related), but for 2010 only sporadic cases were included. Now all years include sporadic and outbreak related *Salmonella* cases.

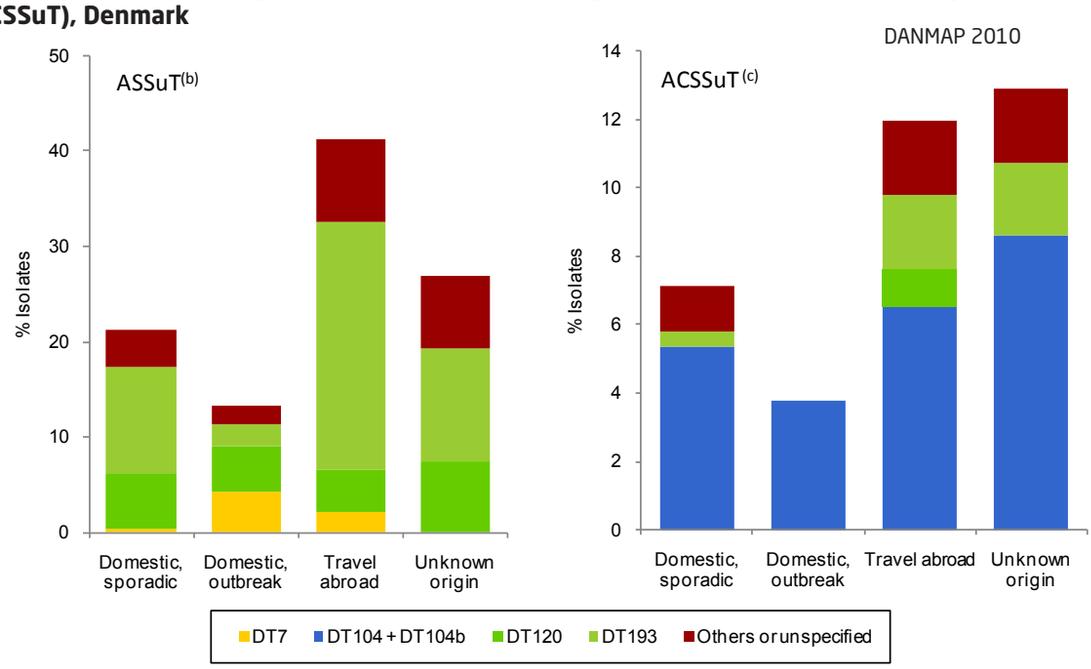
Table 6.2. Resistance (%) in *Salmonella* Typhimurium[a] from human cases reported[b] as domestically aquired (sporadic or outbreak related), associated with travel abroad or as of unknown origin, Denmark

DANMAP 2010

Antimicrobial agent	Domestic sporadic %	Domestic outbreak %	Travel abroad %	Unknown origin %
Tetracycline	36	21	59	54
Chloramphenicol	7	4	13	14
Florfenicol	6	4	12	11
Ampicillin	42	78	60	58
Ceftiofur	0	0	3	1
Cefotaxime	-	-	-	-
Sulfonamide	44	87	64	63
Trimethoprim	5	1	8	8
Apramycin	1	0	0	0
Gentamicin	1	0	3	2
Neomycin	4	0	2	4
Spectinomycin	9	4	14	14
Streptomycin	43	87	59	58
Ciprofloxacin[c]	4	4	14	15
Nalidixic acid	2	4	8	13
Colistin	1	0	0	1
Number of isolates	227	212	95	95

a) Include the monophasic *S.* Typhimurium isolates
b) The isolate was categorized as 'domestically acquired' if the patient did not travel one week prior to the infection, and it was characterized as 'travel abroad reported' if the patient travelled one week prior to the infection
c) The higher occurrence of resistance to ciprofloxacin compared to resistance to nalidixic acid in 2010 was in part due to *qnr* genes

Figure 6.5. Proportional distibution of phage types among *Salmonella* Typimurium isolates[a] from human cases resistant to ampicillin, streptomycin, sulfonamide and tetracycline without or with chloramphenicol resistance (ASSuT/ACSSuT), Denmark



a) In 2010, a total of 629 *S.* Typhimurium cases were reported, including the monophasic Typhimurium isolates.
Domestic sporadic: n = 227; Domestic outbreak: n = 212; Travel abroad: n = 95 and Unknown origin: n = 95
b) Isolates with 'ASSuT' can also include resistence to other antimicrobial agents except chloramphenicol
c) Isolates with 'ACSSuT ' all include resistance to chloramphenicol but can also include resistance to other antimicrobial agents

Salmonella Enteritidis in humans

In contrast to the few travel-related cases of *S.* Typhimurium, 60% of the human *S.* Enteritidis cases reported travelling abroad, 18% of *S.* Enteritidis cases were domestically acquired and the remaining 22% had an unknown origin. Of the domestically acquired cases, 97% were considered to be sporadic. These were of phage types PT2, PT8, PT14c, PT15a and RDNC.

The majority of *S.* Enteritidis isolates from domestic sporadic cases (91%), travel-related cases (73%) and cases of unknown origin (78%) were fully sensitive, whereas one (2%) domestic sporadic case, six (3%) travel-related cases and one (1%) case of unknown origin were multi-resistant.

Of the 388 *S.* Enteritidis isolates available, 364 isolates had valid results from susceptibility testing for all antimicrobial agents in the test panel (Table 6.3). Two isolates were part of a domestic outbreak, both resistant to ciprofloxacin and nalidixic acid. A total of 8% of the domestic sporadic isolates were ciprofloxacin and nalidixic acid resistant. In contrast, 21% of the cases reported to be travel-related or of unknown origin were resistant to these two antimicrobial agents.

6.2.3 Attribution of human *S.* Typhimurium infections (ASuT) to sources of animal origin, 2007-2010

Salmonella has been among the most important foodborne pathogen in Denmark in the last decades. To assist prioritization of interventions to reduce the burden of human salmonellosis in the country, the Danish Zoonosis Centre has routinely applied a source attribution model to estimate the contribution of the major animal-food sources to human infections of *Salmonella*.

The principle of the method is to compare the number of human cases caused by different *Salmonella* sero- and phage types with the distribution of the same subtypes isolated from the various animal-food sources. Antimicrobial resistance patterns of *S.* Typhimurium isolates are also included to further distinguish between similar phage types found in animals, food and humans [Annual Report on Zoonoses in Denmark 2010].
In 2010, 642 human *S.* Typhimurium cases were reported, of which 59 (9%) sporadic cases were attributed to domestic pork, 68 (11%) to imported pork, 13 (2%) to imported poultry products, 133 (21%) to international travel, 221 (34%) cases were associated with outbreaks, and 148 (23%) were attributed to "unknown source". In addition to the 59 *S.* Typhimurium sporadic cases attributed to domestic pork, there were 172 *S.* Typhimurium outbreak-related cases attributed to this source.

Among the sporadic *S.* Typhimurium cases attributed to domestic pork, five were caused by isolates with ASuT resistance pattern (resistant to at least ampicillin, sulfonamide and tetracycline but not chloramphenicol and one was caused by a *S.* Typhimurium isolate with ACSuT resistance pattern (resistant to at least ampicillin, sulfonamide, tetracycline and chloramphenicol). In addition, one of the 172 pork related outbreak cases had the ASuT resistance pattern (phage type DTU323); most of the isolates from these outbreak-related cases were resistant to ampicillin, streptomycin and sulfonamide only.

Table 6.3. Resistance (%) in *Salmonella* Enteritidis from human cases reported[a] as domestically aquired (sporadic or outbreak related), associated with travel abroad or as of unknown origin, Denmark DANMAP 2010

	Humans			
Antimicrobial agent	Domestic sporadic %	Domestic outbreak %	Travel abroad %	Unknown origin %
Tetracycline	2	0	5	4
Chloramphenicol	2	0	1	0
Florfenicol	0	0	0	0
Ampicillin	3	0	8	4
Ceftiofur	0	0	0	0
Cefotaxime	-	-	-	-
Sulfonamide	2	0	2	0
Trimethoprim	2	0	2	0
Apramycin	0	0	0	0
Gentamicin	0	0	0	0
Neomycin	0	0	0	0
Spectinomycin	0	0	0	1
Streptomycin	2	0	2	1
Ciprofloxacin	8	100	21	21
Nalidixic acid	8	100	19	21
Colistin	16	0	29	16
Number of isolates	64	2	217	81

a) The isolate was categorized as 'domestically acquired' if the patient did not travel one week prior to the infection, and it was characterized as 'travel abroad reported' if the patient travelled one week prior to the infection

Among the 68 sporadic *S.* Typhimurium cases attributed to imported pork, 41 were caused by *S.* Typhimurium ASuT and 10 were caused by *S.* Typhimurium ACSuT. One *S.* Typhimurium DT193 with ASuT resistance pattern was attributed to imported turkey.

The *Salmonella* source attribution model does not include results of streptomycin susceptibility testing and therefore the results relate to ASuT only. However, the majority of the attributed cases with the ASuT +/- Chloramphenicol was also resistant to streptomycin.

An analysis of the number of sporadic *S.* Typhimurium ASuT and ACSuT cases attributed to Danish and imported pork during the period 2007 through 2010 showed fluctuations in the relative importance of sources and phage types over the years (Figure 6.6). The most relevant changes were observed in the contribution of Danish and imported pork to human sporadic cases caused by both resistance patterns. The number of sporadic ASuT cases attributed to Danish pork increased markedly from 2007 to 2008, where cases were caused by DT120 and unspecified phage types in both years. The contribution of domestic pork subsequently decreased, and in 2010 was reduced to five cases as described above. In contrast, the number of sporadic ASuT cases attributed to imported pork increased eight-fold from 2007 to 2010. The most evident increase was observed in cases caused by phage types DT193 but also DT120 (Figure 6.6).

Among sporadic cases caused by *S.* Typhimurium with ACSuT resistance, the number of cases attributed to domestic pork also decreased substantially, from 14 in 2007 to one in 2010, whereas the number of cases attributed to imported pork decreased from 2007 to 2009, but increased again in 2010 where nine DT104 or DT104b cases and one non-typeable isolate were attributed to this source (Figure 6.6).

During the period from 2007 through 2010, one sporadic ASuT case was attributed to imported broiler meat (95% Credibility Interval (CI): 0 - 3 cases), whereas three sporadic ASuT cases (95% CI: 0 - 9) and two sporadic ACSuT cases (95% CI: 0-4) were attributed to imported turkey. In 2007, five sporadic ASuT cases were attributed to Danish beef, and in 2009, one sporadic ASuT case was attributed to Danish broiler meat. No ACSuT cases were attributed to Danish beef or broiler meat during this time period.

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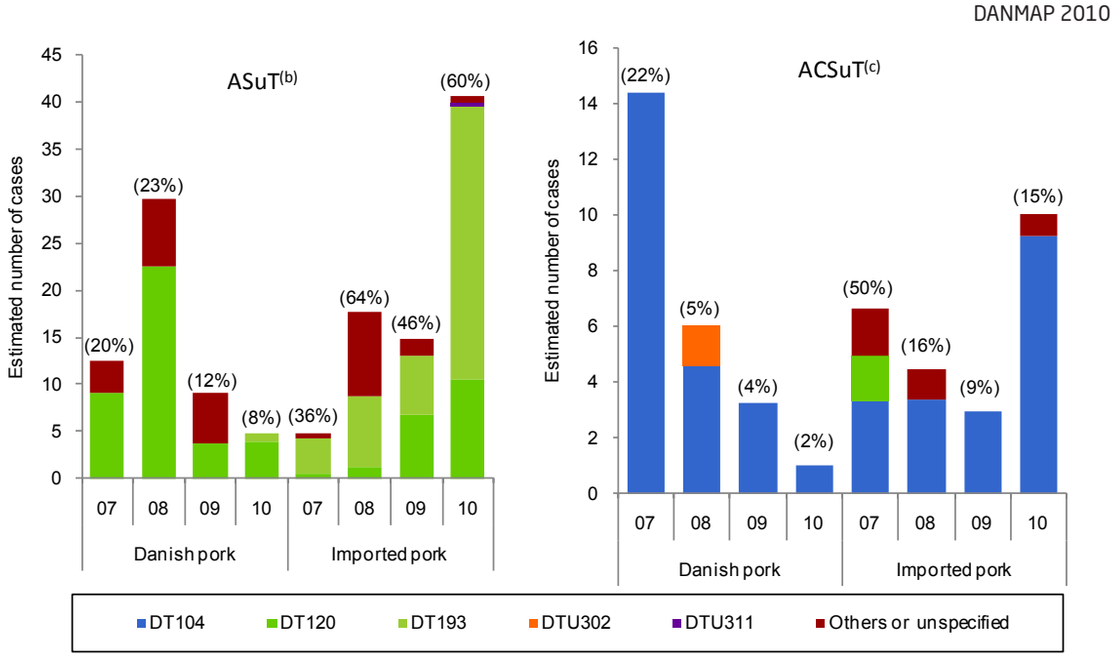
Figure 6.6. Estimated number of sporadic human *Salmonella* Typimurium cases[a] resistant to ampicillin, sulfonamide and tetracycline[b] without or with chloramphenicol (ASuT/ACSuT), attributed to Danish and imported pork by phage type, Denmark



a) Numbers in parentheses indicate the proportion of ASuT/ACSuT cases of all sporadic domestic *S.* Typhimurium cases attributed to Danish and Imported pork during the period. The total number of sporadic domestic *Salmonella* cases in 2007: n = 776; 2008: n = 1,235; 2009: n = 802 and 2010: n = 592

b) Isolates with 'ASuT' can also include resistance to other antimicrobial agents except chloramphenicol

c) Isolates with 'ACSuT' all include resistance to chloramphenicol but can also include resistance to other antimicrobial agents

6.2 *Campylobacter*

Since 2005, *Campylobacter* have been the most commonly reported cause of gastrointestinal bacterial infections in humans in Denmark as well as in the European Union.

Human campylobacteriosis is caused by thermotolerant *Campylobacter* spp. The species most commonly associated with human infections are *C. jejuni* followed by *C. coli,* but other species are also known to cause infections in humans. In Denmark, 85-95% of the human campylobacteriosis cases are caused by *C. jejuni*. Thermotolerant *Campylobacter* are widespread in nature and the most important reservoirs are the alimentary tract of wild and domesticated birds and mammals. They are prevalent in production animals such as poultry, cattle, pigs and sheep; in pets, including dogs and cats; in wild birds and environmental water sources. The bacteria can readily contaminate various foodstuffs. Among sporadic human cases, contact with live poultry, consumption of poultry meat, drinking water from untreated water sources, and contact with pets and other animals have been identified as the major sources of infection.

6.2.1 *Campylobacter jejuni*

Production animals
In 2010, samples from animals were collected at slaughter for the DANMAP programme and all further testing was performed at the National Food Institute. Only one isolate per farm was included in the report. *C. jejuni* from pigs were not included because very few *C. jejuni* isolates were found in pigs.

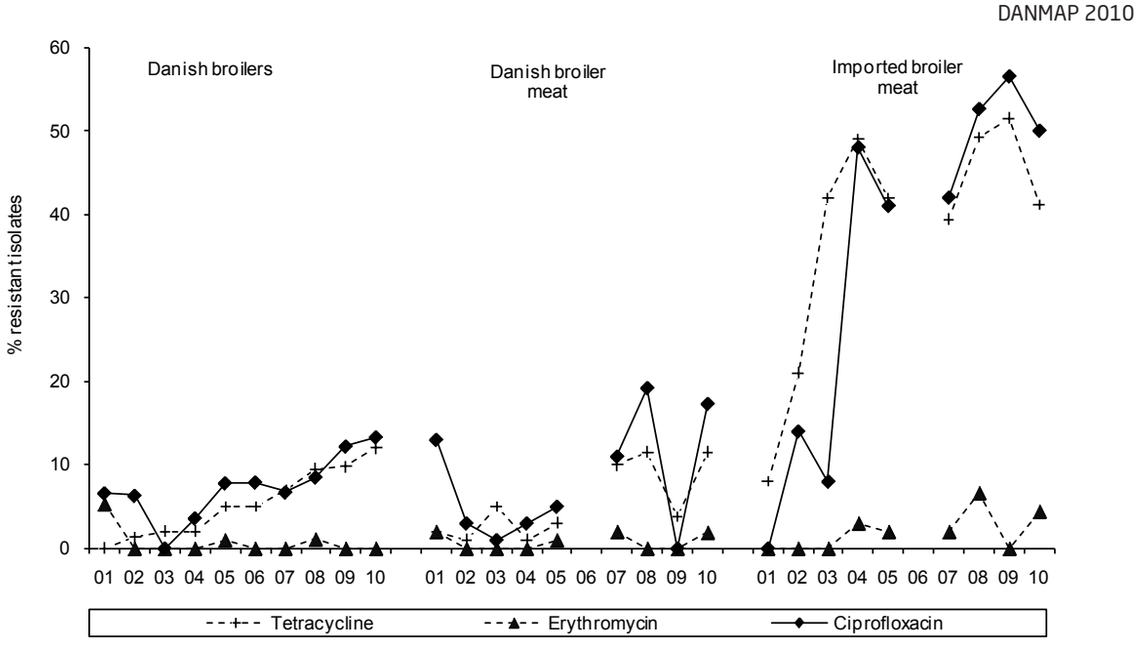
For broilers, 382 flocks were sampled representing 169 different farms. A total of 41 *C. jejuni* were isolated and susceptibility tested. Of these, 75% were fully sensitive to the antimicrobial agents tested.

In isolates from Danish broilers, the highest levels of resistance were found for ciprofloxacin (19% in 2010 compared to 13% in 2009) and tetracycline (17% in 2010 compared to 12% in 2009). An almost continuous increase in resistance to ciprofloxacin and tetracycline has been observed over the last decade from less than 10% resistant isolates in 2000 to 19% and 17% in 2010, respectively (Figure 6.7).

Following an increase in consumption of fluoroquinolones for rearing and parent flocks in 2009, fluoroquinolones were not used in these flocks in 2010. A decrease in consumption was also observed for amoxicillin, penicillins, sulfonamides and macrolides in rearing flocks for the broiler production. However, in broiler flocks, the consumption of tetracycline, which increased considerably from 2008 to 2009, continued to increase in 2010. Second to amoxicillin, tetracycline was the most commonly used antimicrobial agent in Danish broilers in 2009 and 2010; this is a potential explanation for the increasing tetracycline resistance in *C. jejuni* from Danish broilers. Furthermore, from 2009 to 2010, the consumption of amoxicillin, penicillins and sulfonamides in broiler flocks also increased (Figure 4.8).

For cattle, 216 samples were analysed for *Campylobacter* and 98 randomly selected *C. jejuni* isolates were susceptibility tested; 75% of the *C. jejuni* isolates from cattle were fully sensitive to the antimicrobial agents tested.

Figure 6.7. Resistance (%) in *Campylobacter jejuni* from broilers, Danish broiler meat and imported broiler meat, Denmark

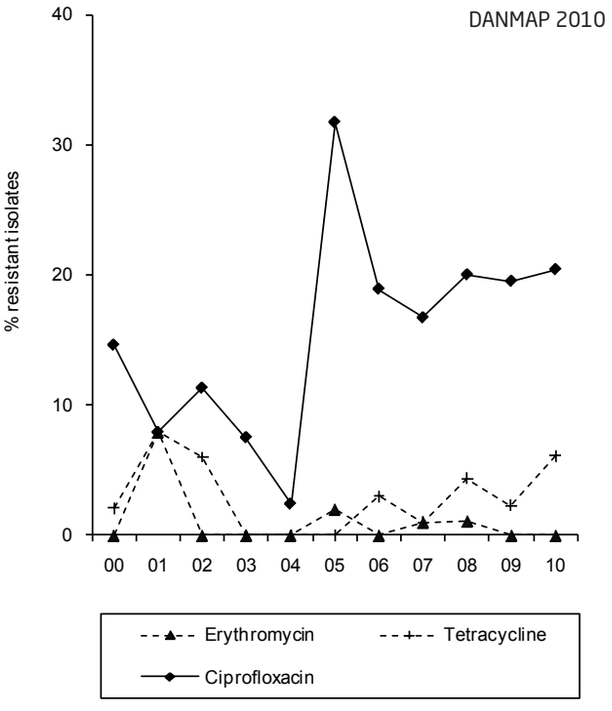


Resistance to ciprofloxacin among *C. jejuni* from cattle has remained virtually unchanged since 2008, at a level around 20% (Figure 6.8). As described in previous DANMAP reports, a significant increase in the level of fluoroquinolone resistance occurred in 2005 despite low consumption of fluoroquinolones in cattle since 2003. As in previous years, only few of the fluoroquinolone resistant isolates were also resistant to tetracycline in 2010, indicating that co-selection by tetracycline (one of the major drugs for treatment of calves) was not the explanation for the high occurrence of fluoroquinolone resistance. It has been discussed [DANMAP 2007] that clonal spread, particularly between farms, could be an explanation for the observed resistance to fluoroquinolones. Initially, the fluoroquinolone resistant *C. jejuni* isolates were obtained from cattle farms in Southern Jutland, but the occurrence of resistance has been moving north from 2007 to 2010 and in 2010, 85% of the ciprofloxacin resistant *C. jejuni* isolates originated from farms in Jutland, with a high prevalence in Northern Jutland.

Since 2005, the resistance to tetracycline in isolates obtained from cattle has increased from 0% to 6% and the resistance is now at the same level as in 2002 (Figure 6.8).

MIC distributions among *C. jejuni* from cattle and broilers in 2010 are shown in Appendix 1 (Table AP1.14).

Figure 6.8. Resistance (%) in *Campylobacter jejuni* from cattle, Denmark



Meat

The Danish Veterinary and Food Administration (DVFA) collected samples from broiler meat, sold at wholesale and retail outlets, for *Campylobacter* testing. In 2010, isolates from food were species identified in the regional laboratories of DVFA and susceptibility tested at the National Food Institute.

From broiler meat, all isolates verified as *C. jejuni* (120 isolates; 52 Danish, 68 imported) were susceptibility tested. Only one isolate per sample was reported (Table 6.4). Among the Danish isolates, 75% were fully sensitive to the antimicrobial agents tested, whereas only 37% of the isolates from imported meat were fully sensitive.

The observed resistance to ciprofloxacin and tetracycline has fluctuated over the last three years, and in 2010 the resistance increased, returning to the same levels as reported in 2008. Hence, resistance to ciprofloxacin in *C. jejuni* isolates from Danish broiler meat increased significantly from 0% in 2009 to 17% in 2010. However, it should be noted that only half as many samples were tested in 2009 compared to 2010.

As in previous years, resistance to ciprofloxacin and tetracycline was significantly higher in *C. jejuni* from imported broiler meat compared to Danish broiler meat (Figure 6.7). In 2009, a significant decrease in erythromycin resistance among the tested *C. jejuni* isolates from imported broiler meat was observed.

Erythromycin resistance has remained at a very low level in both domestic and imported broiler meat for almost a decade.

MIC distributions among *C. jejuni* from broiler meat in 2010 are shown in Appendix 1 (Table AP1.16).

Table 6.4. Resistance (%) in *Campylobacter jejuni* from animals, Danish broiler meat, imported broiler meat and in domestic and travel related human cases, Denmark

DANMAP 2010

Antimicroial agent	Cattle	Broilers	Broiler meat		Humans	
	Danish	Danish	Danish	Imported	Domestically acquired	"Travel abroad"
	%	%	%	%	%	%
Tetracycline	6	17	12	41	13	57
Chloramphenicol	0	0	0	0	0	0
Erythromycin	0	0	2	4	0	0
Gentamicin	0	0	0	0	0	0
Streptomycin	1	2	2	0	2	2
Ciprofloxacin	20	20	17	50	25	80
Nalidixic acid	20	17	13	50	23	80
Number of isolates	98	41	52	68	52	46

Humans

Campylobacter continued to be the most frequent cause of bacterial intestinal infections in 2010. A total of 4,035 human laboratory confirmed cases of campylobacteriosis were reported (73 per 100,000 inhabitants), representing an increase of 20% compared to 2009 [Annual report on Zoonoses in Denmark 2010]. For the surveillance of antimicrobial resistance, the former counties of Northern Jutland, Funen and Roskilde were selected, representing approximately 25% of all cases in Denmark in 2010.

Since 2007, SSI has collected information on travel history through phone interviews from all *Campylobacter* patients residing in the above mentioned counties. Patients were asked about the date of disease onset and whether they had travelled abroad within a seven-day period prior to the onset of disease. Furthermore, patients who had been abroad were asked about their destinations. Cases were categorised as "domestically acquired" if the patients had not travelled within the last week prior to the onset of infection.

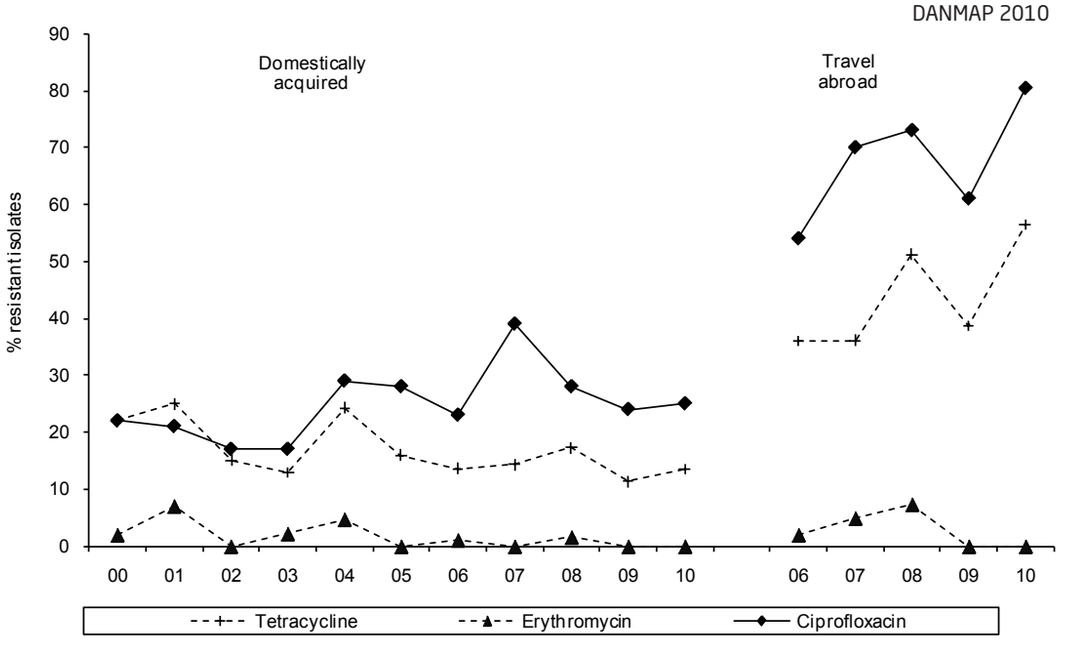
In 2010, 141 *Campylobacter jejuni* isolates were submitted to SSI for susceptibility testing continuously over the year. The isolates were randomly selected from all *Campylobacter* isolated from stool samples in the three counties mentioned above. Among the tested isolates, 46 (33%) were from travel-associated cases and 52 (35%) were considered to be domestically acquired. For the remaining 43 cases, it was not known whether they were acquired domestically or abroad. Among the isolates from domestically acquired infections, 67% were fully sensitive to the antimicrobial agents tested, while the percentage of fully sensitive isolates was much lower, 17%, among isolates from travel associated cases (Table 6.4).

MIC distributions and the occurrence of antimicrobial resistance among *C. jejuni* from domestically acquired human cases and human cases associated with travel are shown in Appendix 1 (Table AP1.17).

In 2010, the level of resistance to ciprofloxacin in *C. jejuni* isolates from domestically acquired infections remained in between the level of resistance for isolates obtained from Danish broiler meat and imported broiler meat. The consumption of imported broiler meat has continued to increase in Denmark, from 17% in 2003 to 33% in 2006 to 45% in 2010 [Annual report on Zoonoses in Denmark 2010]. It is likely that imported broiler meat contribute to the relatively high occurrence of ciprofloxacin resistance (25%) in *C. jejuni* isolates from domestically acquired human infections.

The occurrence of resistance to ciprofloxacin and tetracycline continued to be significantly higher in travel associated *C. jejuni* isolates (80% and 57%, respectively) compared to isolates from domestically acquired infections (25% and 14%, respectively) (Figure 6.9). For the other antimicrobial agents tested, no significant differences in resistance levels were observed. Ciprofloxacin or other fluoroquinolones are often used for empiric treatment of adults with severe bacterial gastroenteritis. Fluoroquinolones are also used in animal husbandry. However, in Denmark the consumption of fluoroquinolones in animal husbandry has been restricted since 2002. Travelling to or consuming meat from countries where fluoroquinolone restrictions are not implemented can be associated with a higher risk of acquiring infection with ciprofloxacin resistant *C. jejuni*.

Figure 6.9 Resistance (%) in *Campylobacter jejuni* from human cases, Denmark



6.2.2 *Campylobacter coli*

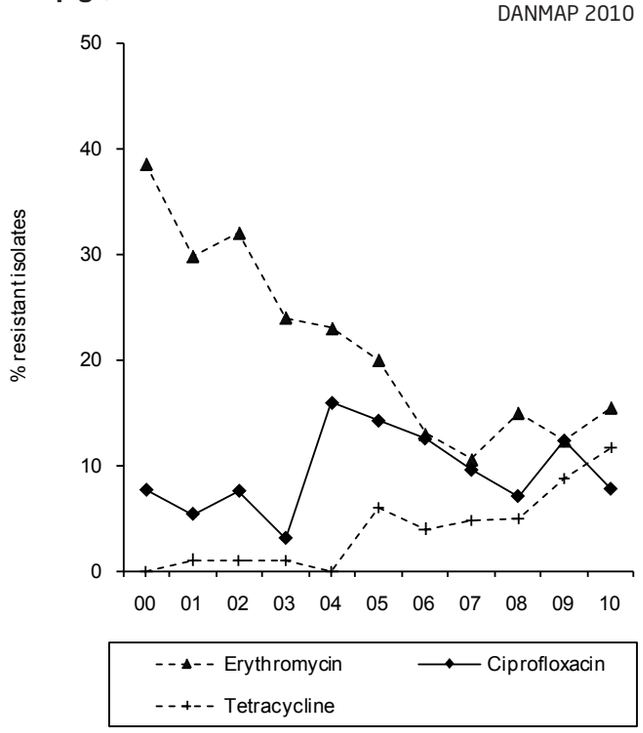
Production animals
For animals, only antimicrobial resistance among *C. coli* isolates from pigs is reported in the DANMAP report 2010. MIC distributions and the occurrence of antimicrobial resistance among *C. coli* from pigs are shown in Appendix 1 (Table AP1.13).

In 2010, 269 samples from pigs were analysed for *Campylobacter*; 103 randomly selected *C. coli* isolates were susceptibility tested, and 26% were found fully sensitive. As in the previous three years, no significant changes in fluoroquinolone resistance were observed among *C. coli* from pigs from 2009 to 2010. Fluoroquinolone resistance was detected in approximately 8% of the tested isolates (Figure 6.10) despite low consumption of fluoroquinolones in pigs since 2003 (Table AP1.1).

In 2010, erythromycin resistance in *C. coli* from pigs was 15%. A continuous decrease in erythromycin resistance in *C. coli* was observed after withdrawal of the growth promoter tylosin from the Danish pig production in 1998–1999. However, in the past years, the level of resistance has not changed significantly and since 2006 the level of resistance has been 10-15% (Figure 6.10). In contrast, an increasing trend has been observed in the occurrence of resistance to tetracycline for *C. coli* from Danish pigs over the past decade, especially during 2004–2010. Overall, the consumption of tetracycline has also increased from 2001 to 2009 (Table AP1.1).

Figure 6.10. Resistance (%) in *Campylobacter coli* from pigs, Denmark

DANMAP 2010



In 2010, streptomycin resistant *C. coli* isolates from pigs increased significantly from 48% to 63%. While this increase may reflect that the isolates coincidentally originated from producers with high prevalence of diseases typically treated with streptomycin (limb, joint, CNS and skin), it is noteworthy that the consumption of penicillin-streptomycin combinations for finisher pigs also increased during this period. However, it should be noted that the increase in streptomycin consumption represents only a very small fraction of the total consumption (Table AP1.1).

Meat
Only one isolate per sample was susceptibility tested and reported. In 2010, a total of 47 C. *coli* isolates from broiler meat were susceptibility tested, 20 isolates were obtained from Danish broiler meat and 27 were obtained from imported broiler meat.

Among the Danish isolates, 11 (55%) were found fully sensitive, while only two (7%) isolates from imported broiler meat were fully sensitive to the antimicrobial agents tested. Significant differences were observed in resistance depending on the origin of the meat (Danish/imported), the MIC values are shown in Appendix 1 (Table AP1.15).

Resistance levels in the isolates from Danish broiler meat were: No resistance to ciprofloxacin or erythromycin, and 35% resistance to tetracycline. The corresponding values for the isolates obtained from imported meat were: 85% resistance to ciprofloxacin, 14% resistance to erythromycin, and 82% resistance to tetracycline. While there were no significant changes observed in the resistance levels in the Danish isolates from 2009 to 2010, this was not the case for the imported isolates. Thus, 85% of the imported isolates were resistant to ciprofloxacin in 2010 compared to 42% in 2009. Similarly, the resistance level for erythromycin increased from 2% to 15% and resistance to tetracycline increased from 51% to 81%.

Humans
No *C. coli* isolates from human cases were included in the DANMAP report 2010, since there was only a small number of *C. coli* among the *Campylobacter* isolates received at SSI.

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Textbox 3

Occurrence of *Clostridium difficile* in Danish pig farms, and in cattle and broilers at slaughter

Background: *Clostridium difficile* is increasingly causing infection in humans and have lately caused outbreaks at hospitals in Denmark and other countries as well [DANMAP 2009]. Especially, the virulent *C. difficile* 027 is causing severe infections. Even though, *C. difficile* can be isolated from animals and meat, its role as a zoonosis is not fully understood [Rupnik. 2007. Clin Microbiol Infect. 13: 457-9]. The aim of this study was to investigate the occurrence of *C. difficile* in pig farms, and in cattle and broilers at slaughter to determine if types more likely to cause disease in humans were present in animals.

Materials and methods: During June through November 2010, 99 stool samples from slaughter pig pens at 99 farms, 192 faecal samples from cattle at slaughter and 197 pools of cloacal swabs of broilers at slaughter were collected and tested for the presence of *C. difficile*. The pools of cloacal swabs were added to 2.5 ml of 0.9% NaCl and mixed. One milliliter of cells suspended in the 0.9% NaCl or 1 gram of faecal sample was added to 9 ml CDMN broth supplemented with 0.1% Sodium taurocholate and incubated anaerobically at 37°C for 7 days. Two ml were transferred to 2 ml 99% ethanol and left at room temperature for one hour. After centrifugation, 10 µl of pellet was transferred to a CDMN agar plate. The plates were incubated for 44 to 48 hours anaerobically at 37°C. Presumptive *C. difficile* were re-streaked at CDMN agar plates and the presence of *C. difficile* verified by PCR detection of toxin genes (*tcdA*, *tcdB*, binary toxin) as previously described [Persson *et al*. 2008. Clin Microbiol Infect. 14: 1057-64]. Isolates positive for all three toxin genes were furthermore PCR ribotyped and tested for deletions in *tcdC*. All isolates containing toxin genes were tested for resistance to clindamyin (1-16mg/l), erythromycin (0.5-8 mg/l), metronidazole (4-64 mg/l), moxifloxacin (1-16 mg/l), and vancomycin (4-64 mg/l) following CLSI guidelines (see Table 1 for resistance breakpoints).

Results and discussion: Fifteen (15%) of the pig farm samples were positive for *C. difficile*. All of the isolates were tested for toxin genes and 73% had all three toxin genes whereas 27% had *tcdA* and *tcdB*. Twenty-nine (15%) of the cattle samples were positive for *C. difficile*. All of the isolates were tested for toxin genes and 24% contained all three genes, 69% contained *tcdA* and *tcdB,* and 7% contained only *tcdA*. Six broiler flocks (3%) were positive for *C. difficile* and all six isolates contained *tcdA* and *tcdB*. Six of the eighteen isolates from pigs and cattle that contained all three toxin genes belonged to PCR ribotype 078 (two from cattle and four from pigs), the rest belonged to PCR ribotypes rarely or not previously found in humans in Denmark. Fifteen of the isolates had a 39 bp deletion in *tcdC* and three had a 54 bp deletion in *tcdC*. These three isolates all belonged to the same PCR ribotype (named DK136) and originated from cattle. One human case with PCR ribotype DK136 has been reported in Denmark. More than half of the isolates contained *tcdA* and *tcdB,* and approximately one-third of the human cases in 2009 were caused by *C. difficile* with these toxins [DANMAP 2009]. Types found in animals and human cases cannot be directly compared since the human isolates are selected for typing based on three criteria used to screen for the 027 type: Resistance to moxifloxacin, if the cases have severe clinical manifestations, or if cases are suspected to be part of an outbreak. The most common PCR ribotypes in humans in Denmark are 027, 078, 066 and 023.

The MIC distributions of five antimicrobial agents are presented in Table 1. Most isolates were resistant to clindamycin (87%) and all isolates were susceptible to vancomycin and metronidazole. One isolate from pigs and one isolate from cattle were resistant to erythromycin. Moreover, one isolate from pigs was resistant to moxifloxacin. This isolate contained all three toxin genes and belonged to a PCR ribotype not previously found in humans (named DK135). Resistance to moxifloxacin is one out of three criteria used to screen for type 027 in Danish hospitals (see above) [DANMAP 2009].

In conclusion, the finding of *C. difficile* 078 in pigs is not surprising since this type is known to be common among pigs. However, other types may have a potential to cause severe disease in humans, as isolates with all three toxin genes and deletion in *tcdC* were found. Moreover, the importance of *C. difficile* with *tcdA* and *tcdB* in animals should be further investigated.

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Table 1. Resistance (%) in *C. difficile* from broilers (n = 6), cattle (n = 26) and pigs (n = 14)

DANMAP 2010

Antimicrobial agent	Animal type	% Resistant	Distribution (%) of MICs							
			0.5	1	2	4	8	16	32	64 mg/l
Clindamycin	Broilers	100					83	17		
	Cattle	81		8	8	4	65	8	8	
	Pigs	93			7		57	29	7	
Erythromycin	Broilers	0		66	33					
	Cattle	4	27	69				4		
	Pigs	7	7	79	7			7		
Metromidazole	Broilers	0				100				
	Cattle	0				100				
	Pigs	0				100				
Moxifloxacin	Broilers	0		83	17					
	Cattle	0		85	12	4				
	Pigs	7		93				7		
Vancomycin	Broilers	0				100				
	Cattle	0				100				
	Pigs	0				100				

Note The resistance breakpoints are indicated with black vertical lines

7 RESISTANCE IN INDICATOR BACTERIA

7. Resistance in indicator bacteria

Indicator bacteria (*Enterococcus faecium, Enterococcus faecalis* and *Escherichia coli*) have been included in the DANMAP programme since 1995. These bacteria are included since they can be isolated from faecal samples from animals and humans. During slaughter of production animals, meat can be contaminated with enterococci and *E. coli*. Furthermore, enterococci and *E. coli* easily develop antimicrobial resistance in response to selective pressure.

Most of the antimicrobial agents which have been used for growth promotion in Denmark (banned in 1998) had effect on Gram-positive bacteria like enterococci; especially *E. faecium*. Today, many of the antimicrobial agents used in veterinary clinical therapy are broad spectrum and are mainly active against Gram-negative bacteria, such as *Salmonella* and *E. coli*.
Enterococci are still included in the DANMAP programme to follow the persistence of resistance after the ban of growth promoters. *E. coli* is included in the programme, since they are more often isolated from faecal samples and meat than *Salmonella*, and are therefore a better indicator for occurrence of antimicrobial resistance.

7.1 Enterococci

Enterococcus faecium and *Enterococcus faecalis* were isolated from faecal samples from pigs and broilers. No enterococci were isolated from cattle. All samples included in the DANMAP surveillance programme were collected at the time of slaughter. Enterococci from food originated from meat sold at wholesale and retail outlets, collected randomly in all regions of Denmark by the Danish Veterinary and Food Administration Regional Laboratories in centrally coordinated programmes. The identification and susceptibility testing was done at the National Food Institute. The MIC distributions and occurrence of resistance among *E. faecium* and *E. faecalis* are presented in Appendix 1 (Tables AP1.18, AP1.19, AP1.20 and AP1.21).

7.1.1 *Enterococcus faecium* in production animals

E. faecium was isolated from 18% (136/738) of the samples from pigs and 44% (169/382) of the samples from broilers. Only one isolate per farm was included and a randomly selected subsample of 133 and 119 isolates from pigs and broilers, respectively, were susceptibility tested and reported (Table 7.1).

Pigs

The highest occurrence of resistance was found for tetracycline (51%), followed by streptomycin (35%), erythromycin (27%) and kanamycin (23%) (Table 7.1). Both streptomycin and kanamycin belong to the aminoglycosides. From 2009 to 2010, significant decreases in prevalence of antimicrobial resistance among isolated *E. faecium* from pigs were seen for tetracycline, penicillin, ampicillin and streptomycin. Both ampicillin and penicillin belong to the beta-

Table 7.1. Resistance (%) in Enterococcus faecium from animals and meat of Danish and imported origin, Denmark

DANMAP 2010

Antimicrobial agent	Broilers	Pigs	Broiler meat		Beef meat	Pork meat
	Danish	Danish	Danish	Imported	Danish	Danish
	%	%	%	%	%	%
Tetracycline	6	51	10	43	10	17
Tigecycline	0	0	0	0	0	0
Chloramphenicol	0	0	0	0	0	0
Penicillin	1	3	1	26	0	3
Ampicillin	0	2	1	25	0	0
Erythromycin	26	27	21	63	5	31
Gentamicin	0	0	0	0	0	0
Kanamycin	0	23	1	20	0	3
Streptomycin	1	35	3	37	0	7
Ciprofloxacin	-	-	0	0	0	0
Vancomycin	0	1	1	0	0	0
Quinupristin/dalfopristin	0	2	1	9	0	0
Avilamycin	0	0	-	-	-	-
Salinomycin	53	0	37	10	0	0
Linezolid	0	0	0	0	0	0
Teicoplanin	-	-	1	0	0	0
Number of isolates	119	133	145	107	20	29

lactams. The reduced prevalence of tetracycline resistance can be related to the reduced usage of tetracycline, especially after July 1st 2010 (see chapter 4.3). The reduction in resistance to the beta-lactams (ampicillin and penicillin), however, can be explained partly by the high peak observed in 2009 and partly by the fact that the epidemiological cut-off value recommended by EUCAST does not clearly separate the sensitive population from the resistant, and variation in methodology can therefore lead to large changes in detected prevalence. For streptomycin resistance, which has gradually increased over the last decade without increased consumption, 95% of the streptomycin resistant isolates were also resistant to tetracycline, indicating co-resistance between these antimicrobials and that the reduced consumption of tetracycline could have resulted in lower prevalence of streptomycin resistance. Resistance to vancomycin and quinupristin/dalfopristin still prevails at a low level among *E. faecium* isolated from pigs.

Broilers
The highest occurrence of resistance was found for salinomycin (53%) followed by erythromycin (23%) and tetracycline (6%) (Table 7.1). Salinomycin is widely used as a coccidiostat in the broiler production. Presently no cut-off value is recommended by EUCAST. The used value is equivalent to the one used in DANMAP 2009. From 2009 to 2010, a significant decrease in prevalence of antimicrobial resistance among *E. faecium* isolated from broilers were seen for tetracycline, penicillin, ampicillin, quinupristin/dalfopristin, and avilamycin. Resistance to the growth promoter avilamycin was not detected for the first time since the ban, but the significantly lower occurrence could, as for quinupristin/dalfopristin, be a result of the very low number of tested isolates in 2009 (n=19) compared to the 199 isolates tested in 2010. The reduced prevalence of resistance to tetracycline, penicillin and ampicillin may be explained as for *E. faecium* isolated from pigs.

Using a selective enrichment method, vancomycin resistant *E. faecium* could be detected in 47% of the faecal samples even though avoparcin (glycopeptide) has been banned since 1995 (Textbox 5).

7.1.2 *Enterococcus faecium* in meat

For Danish meat, *E. faecium* was isolated, from 16% (29/184) of the pork samples, 78% (145/187) of the broiler meat samples and 17% (20/118) of the beef samples. For imported meat, *E. faecium* was isolated from 47% (107/226) of the broiler meat samples. Only one isolate per sample was susceptibility tested and reported (Table 7.1).

The level of resistances in 2010 was comparable to the levels observed in 2009 for Danish pork and broiler meat as well as imported broiler meat. When comparing *E. faecium* isolates from Danish and imported broiler meat, a significantly higher occurrence of resistance to ampicillin, erythromycin, kanamycin, penicillin, streptomycin, quinupristin/dalfopristin and tetracycline was found in isolates from imported broiler meat, while higher occurrence of resistance to salinomycin was found among the isolates from Danish broiler meat.

Significantly lower occurrence of resistance was found for tetracycline, kanamycin and streptomycin in *E. faecium* isolates from Danish pork compared to isolates from Danish pigs. Significantly lower occurrence of resistance to salinomycin was found in isolates from Danish broiler meat when compared to Danish broilers.

7.1.3 *Enterococcus faecalis* in production animals

E. faecalis was isolated from 23% (167/738) of the samples from pigs and 44% (169/382) of the samples from broilers. Only one isolate per farm was included and a randomly selected subsample of 157 and 112 isolates from pigs and broilers, respectively, were susceptibility tested and reported (Table 7.2).

Pigs
The highest occurrence of resistance was found for tetracycline (78%) followed by erythromycin (44%), streptomycin (28%) and kanamycin (21%) (Table 7.2). From 2009 to 2010, a significant decrease in prevalence of antimicrobial resistance to tetracycline was detected as could be explained by reduced usage of tetracycline. Using a selctive enrichment method high-level gentamicin resistant (HLGR) *E. faecalis* was detected in 11% of the faecal samples from pigs. A recent study has shown a possible zoonotic link between HLGR *E. faecalis* from pigs and HLGR *E. faecalis* isolated from endocaditis patients (Textbox 4).

Broilers
The highest occurrence of resistance was observed for tetracycline (26%) followed by erythromycin (25%) and streptomycin (4%) (Table 7.2). No resistance to salinomycin was detected. From 2009 to 2010, significant decrease in prevalence of antimicrobial resistance was seen for tetracycline, erythromycin, streptomycin and salinomycin. Prevalence of tetracycline resistance is propably reduced as a consequence of reduced consumption.

As for streptomycin resistance among *E. faecium* isolated from pigs, high (100%) co-resistance between streptomycin and tetracycline was detected (only four isolates). No clear co-resistance between erythromycin and tetracycline could be detected. Only 55% of the erythromycin resistant isolates were tetracycline resistant, but since only a limited number of *E. faecalis* (n=19) was tested in 2009, compared to the 119 in 2010. The observed statistical significant difference for erythromycin and salinomycin could be reflected in this fact.

7.1.4 *Enterococcus faecalis* in meat

E. faecalis was isolated from 46% (84/184) of Danish pork samples, 32% (59/187) of the Danish broiler meat samples and 23% (27/118) of the Danish beef meat samples. For imported meat, *E. faecalis* was isolated

from 52% (91/175) of the pork samples, 46% (104/226) of the broiler meat samples and 36% (36/99) of the beef samples. Only one isolate per sample was susceptibility tested and reported (Table 7.2).

Compared to 2009, a significantly lower occurrence of resistance to erythromycin was observed among isolates from Danish pork and a significantly lower occurrence of tetracycline resistance was found among isolates from imported pork. Compared to Danish pork, a significantly higher occurrence of resistance to tetracycline was found in *E. faecalis* from imported pork.

The levels of resistances in 2010 was comparable to the levels observed in 2009 for both Danish and imported broiler meat; except for tetracycline, where lower occurrence was found in Danish broiler meat in 2010 compared to 2009. A significantly higher occurrence of resistance to erythromycin, kanamycin and streptomycin was observed in *E. faecalis* isolates from Danish broiler meat compared to isolates from imported meat. In imported pork, the level of tetracycline resistance was significantly higher than among isolates from Danish pork.

Significantly lower occurrence of resistance was found for tetracycline, chloramphenicol, erythromycin, gentamicin, kanamycin and streptomycin in *E. faecalis* isolates from Danish pork compared to Danish pigs. Significantly higher occurrence of resistance to tetracycline was found in isolates from Danish broiler meat when compared to Danish broilers (Table 7.2).

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Table 7.2. Resistance (%) among *Enterococcus faecalis* from animals and meat of Danish and imported origin, Denmark

DANMAP 2010

Antimicrobial agent	Broilers	Pigs	Broiler meat		Beef		Pork meat	
	Danish	Danish	Danish	Imported	Danish	Imported	Danish	Imported
	%	%	%	%	%	%	%	%
Tetracycline	26	78	46	55	22	19	13	34
Tigecycline	0	0	0	0	0	0	0	0
Chloramphenicol	0	16	2	5	0	3	1	3
Penicillin	0	0	2	0	0	0	0	0
Ampicillin	0	0	2	0	0	0	0	0
Erythromycin	25	44	17	39	0	3	1	5
Gentamicin	1	11	0	1	0	0	1	2
Kanamycin	1	21	0	18	4	3	2	3
Streptomycin	4	28	8	24	4	8	0	4
Ciprofloxacin	-	-	0	1	0	0	0	1
Vancomycin	0	0	0	0	0	0	0	0
Avilamycin	0	0	-	-	-	-	-	-
Salinomycin	0	0	2	0	0	0	0	0
Linezolid	0	0	0	0	0	0	0	0
Teicoplanin	-	-	0	0	0	0	0	0
Number of isolates	112	157	59	104	27	36	84	91

Textbox 4

Danish pigs are a reservoir of High-level gentamicin resistant *Enterococcus faecalis* associated with infective endocarditis in humans

Background: Infective endocarditis is a life-threatening infection that involves the endocardial surface or vascular structures in proximity to the heart. The intrinsic resistance to a number of antimicrobial agents makes enterococcal infective endocarditis cumbersome to treat. For decades, the mainstay of treatment has been the combination of a cell wall-active agent (ampicillin, penicillin, or vancomycin) and gentamicin. High-level resistance to gentamicin hinders, however, the bactericidal activity of such combinations. High-level gentamicin resistant (HLGR) *E. faecalis* has been associated with the hospital setting and prior health care exposure, which suggests the existence of a hospital reservoir. Nevertheless, enterococci are gut commensals in humans and warm-blooded animals and it is therefore conceivable that there may be reservoirs of *E. faecalis* in the community not directly linked to hospitals. During 2000–2002, the proportion of HLGR *E. faecalis* isolates increased from 2% to 6% in the pig population in Denmark, which coincided with the emergence of HLGR *E. faecalis* isolates among infective endocarditis patients in the North Denmark Region. We recently undertook a study to determine whether pigs are a potential source of *E. faecalis* infections in Denmark.

Materials and methods: We compared HLGR *E. faecalis* isolates from Danish pigs (n = 19), Danish pork (n = 1), community-dwelling humans (n = 2) and patients with infective endocarditis (n = 2) by multi-locus sequence typing (MLST) [Larsen *et al.* 2010. Emerg Infect Dis. 16: 682–4].

Results: Twenty-three of the 24 strains belonged to MLST type ST16. The remaining isolate from pigs belonged to MLST type ST35. The 23 isolates with MLST type ST16 were further characterised by pulsed field gel electrophoresis (PFGE). The PFGE patterns clustered into one major clonal group.

Discussion: Our study provided the first evidence of existence of a widespread community reservoir of HLGR ST16 in Danish pigs, which coincided with the emergence of HLGR ST16 isolates among infective endocarditis patients. One isolate was present in pork, which supports foodborne transmission although direct transmission from animals to humans is also possible.

Our findings support the results of a study in the United States that identified HLGR *E. faecalis* isolates with similar PFGE patterns from pork and fecal swabs of outpatients [Donabedian *et al.* 2003. 41: 1109–13]. These pig-related HLGR isolates in the study from the USA as well as our collection of HLGR ST16 isolates carry the *aac(6')Ie-aph(2")Ia* gene encoding gentamicin resistance. The isolates from pigs belonging to ST16 carried pathogenicity island (PAI) genes [Shankar *et al.* 2006. J Clin Microbiol. 44: 4200–3].

These genes are more frequently detected among *E. faecalis* isolates recovered from sites of infection such as blood and urine or from fecal swabs of inpatients when compared with isolates from fecal swabs obtained from healthy humans.

Further studies are required to better understand the human and veterinary epidemiology of this zoonosis. Areas of study should include size of the reservoir in pigs as well as in the hospital and other health care settings and whether or not other animals, immunocompromised persons, or healthy humans constitute a community reservoir of HLGR *E. faecalis* ST16.

Since 2002, we have observed a continuous increase in the proportion of HLGR *E. faecalis* isolates in the pig population in Denmark (from 6% in 2002 to 20% in 2009). With an annual production of >22 million slaughter pigs, Denmark therefore has a large potential reservoir of HLGR ST16. Additional studies investigating the clinical impact of this increase are urgently needed.

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Detection of vancomycin resistant *Enterococcus faecium* in Danish broilers 15 years after the ban of avoparcin

Background: Data on the occurrence of antimicrobial resistance in animal enterococci presented in DANMAP are generated by antimicrobial susceptibility testing of one random isolate per herd. It has previously been shown that this approach leads to lower resistance frequencies compared to antimicrobial selective methods [Heuer *et al.* 2002. Microb. Drug Resist. 8: 133–138], but generally these resistance frequencies are representative for the occurrence in the tested bacterial population [Vieira *et al.* 2008. Antimicrob Agents Chemother. 62: 535–38]. In order to quantify the differences between the results obtained by selective and non-selective methods, a subset of the faecal samples collected from pigs, broilers and cattle in DANMAP 2010 were additionally analysed for the occurrence of ampicillin and vancomycin resistant enterococci by selective enrichment in antimicrobial-containing media.

Materials and methods: Faecal samples from pigs (n = 123), broilers (n = 100) and cattle (n = 100) were enriched in 5 ml of Enterococcus Selective Broth containing ampicillin (16 µg/ml) or vancomycin (16 µg/ml). Following subculture on Slanetz agar containing the same antimicrobial concentration, colonies typical of *E. faecium* and *E. faecalis* were confirmed by species-specific PCR [Dutka-Malen *et al.* 1995. J Clin Microbiol. 33: 24–27]. Vancomycin-resistant *E. faecium* (VREF) was confirmed by PCR detection of *vanA*.

Results: Ampicillin and vancomycin resistance was only found in *E. faecium.* No resistance to these antimicrobial agents was detected in *E. faecalis* isolates from all other tested animal reservoirs (pigs, broilers and cattle).VREF was isolated at high frequency (47%) in samples from broilers, while no resistant isolates were detected using the non-selective method. None of the samples from pigs and cattle were positive for VREF. *E. faecium* resistant to ampicillin was found in all animal species (28% in broilers, 4% in cattle and 2% in pigs).

Discussion and conclusions: The study confirmed marked differences between data generated by selective and non-selective methods for VREF from broilers. The lack of detection by random isolation is due to the relatively low concentration of VREF per gram compared to the total *E. faecium* concentration in broiler faeces. The ratio of vancomycin resistant enterococci to the total enterococci count has previously been reported to range from 0.8% to 4.6% in Norwegian broilers 10 years after the ban of avoparcin [Sørum *et al.* 2006. Appl Environ Microbiol. 72: 516–521], which makes VREF detection nearly impossible by the current method used in DANMAP. Ampicillin resistant *E. faecium* were isolated from nearly one third of the broiler flocks when using the selective enrichment even though ampicillin resistance was not detected in *E. faecium* isolated by the standard DANMAP procedure (see Table 7.1). Resistance to ampicillin and vancomycin was low (≤2%) or absent in samples from pigs and cattle, and no significant differences were observed between the two methods in these animal species.

The reasons for the long persistence of VREF in Danish broilers 15 years after the avoparcin ban are unknown.

The VREF isolates in this study were not multilocus sequenced typed (MLST), but other studies have shown that *E. faecium* isolates of poultry origin cluster together in one cluster (CC9), whereas human *E. faecium* isolates belong to another cluster (CC17) [Willems *et al.* 2005. Emerg Infect Dis. 11: 821–8]. However, as shown by a recent *in vivo* study, *E. faecium* of poultry origin can act as a donor of antimicrobial resistance genes for pathogenic *E. faecium* of human origin [Lester and Hammerum 2010. J Antimicrob Chemother. 65: 1534–6].

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7.2 *Escherichia coli*

E. coli isolates from healthy production animals originated from faecal samples collected for the DANMAP programme at the time of slaughter. For broilers, isolation and susceptibility testing was performed at the National Veterinary Institute and for cattle and pigs at the National Food Institute. *E. coli* from meat originated from meat sampled at wholesale and retail outlets, collected randomly in all regions of Denmark by the Danish Veterinary and Food Administration Regional Laboratories in three centrally coordinated programs. The susceptibility testing was done at the National Food Institute. Samples from healthy humans have not been collected since 2008. See the definition of multi-resistance in appendix 2

7.2.1 Indicator *Escherichia coli* from production animals

E. coli was isolated from 91% (199/219) of the samples from pigs and from 92% (122/133) of the samples from cattle. For broilers, 382 flocks were sampled, representing 153 different farms. Only one isolate was included per farm and a randomly selected subsample of 160, 106 and 118 isolates from pigs, cattle and broilers, respectively, were susceptibility tested and reported.

Among animal species, the level of resistance in indicator *E. coli* was lowest in isolates from cattle in 2010 (Table 7.3). Of the susceptibility tested *E. coli* isolates from cattle, 90% were fully sensitive whereas 6% were found to be multi-resistant. However, among the *E. coli* isolates from broilers and pigs that were susceptibility tested, 57% and 43% were fully sensitive, whereas 11% and 33% were found to be multi-resistant, respectively. Trends in resistance to selected antimicrobial agents in isolates from production animals during 2001–2010 are presented in Figure 7.1. The MIC distributions for 2010 are shown in Appendix 1 (Table AP1.22). In general, the highest resistance level was found in *E. coli* from pigs, except for fluoroquinolone (nalidixic acid and ciprofloxacin) resistance which was higher in isolates from broilers (8%).

The low level of fluoroquinolone resistance in *E. coli* from Danish pigs and cattle probably reflects the low consumption since 2002, when the use in production animals was restricted by law. The highest fluoroquinolone consumption was seen in poultry, decreasing since 2006 however, whereas the ciprofloxacin resistance reached the highest observed level in 2007 (14.5%). However, fluoroquinolone resistance in *E. coli* from broilers, pigs and cattle has not changed significantly over the past decade.

In indicator *E. coli* from broilers, no significant changes in resistance were observed in 2010 compared to 2009 (Table 7.3). The highest occurrence of resistance was seen for ampicillin (21%), while amoxicillin has been the most frequently used antibacterial agent in the broiler production for at least a decade (Figure 4.8).

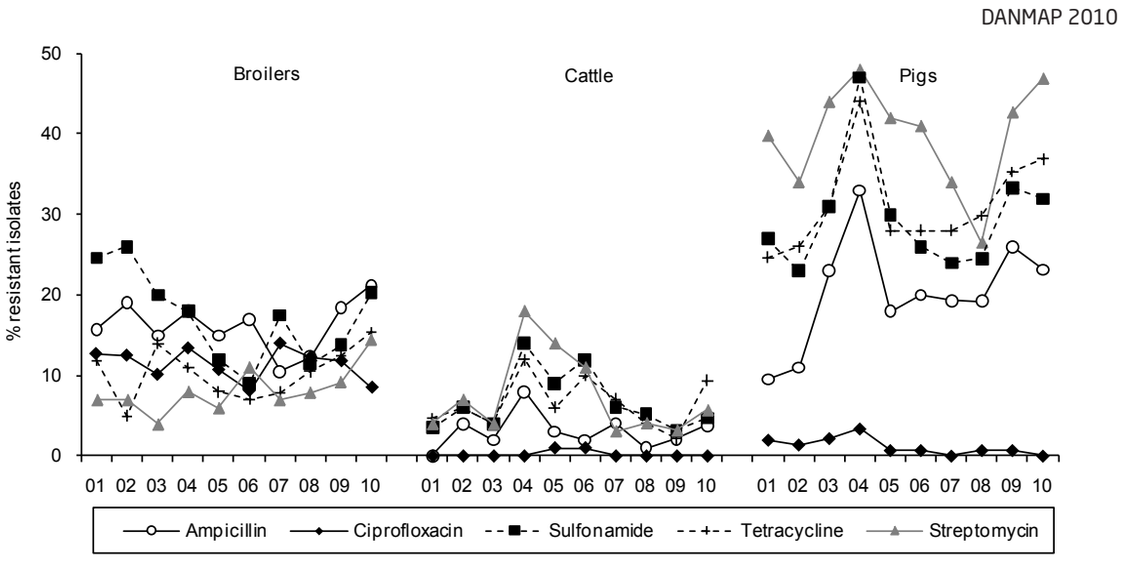
Regarding *E. coli* from pigs, no significant change in occurrence of resistance was observed from 2009 to 2010. For most antimicrobial agents, the consumption was similar in 2009 and 2010. However, important decreases in consumption was seen for tetracyclines (9% per pig produced) and cephalosporins (50% per pig produced) in 2010, related to the second half year (increase in first half year). Since a time lapse is often seen between changes in consumption and

Table 7.3. Resistance (%) in *Escherichia coli* from animals and meat of Danish and imported origin, Denmark

DANMAP 2010

Antimicrobial agent	Broilers	Cattle	Pigs	Broiler meat		Beef		Pork meat	
	Danish	Danish	Danish	Danish	Imported	Danish	Imported	Danish	Imported
	%	%	%	%	%	%	%	%	%
Tetracycline	15	9	37	13	46	3	10	24	56
Chloramphenicol	3	1	4	1	21	0	0	3	8
Florfenicol	1	1	0	0	1	0	0	0	2
Ampicillin	21	4	23	16	58	3	5	24	36
Ceftiofur	0	0	1	1	7	0	3	1	0
Cefotaxime	0	0	1	1	7	0	3	1	0
Sulfonamide	20	5	32	15	56	6	5	19	36
Trimethoprim	8	1	21	4	41	3	3	16	30
Apramycin	1	0	1	0	0	0	0	1	0
Gentamicin	0	0	1	0	3	0	3	1	0
Neomycin	1	1	8	1	10	0	0	3	4
Spectinomycin	5	2	25	4	29	0	3	19	14
Streptomycin	14	6	47	15	46	3	5	38	56
Ciprofloxacin	8	0	0	4	41	0	5	1	4
Nalidixic acid	8	0	0	4	38	0	5	1	4
Colistin	0	0	1	0	6	0	0	0	0
Number of isolates	118	106	160	158	177	32	39	68	50

Figure 7.1. Resistance (%) in indicator *Escherichia coli* from broilers, cattle and pigs, Denmark



a consequent change in resistance in *E. coli* [Jensen *et al*. 2006. J Antimicrob Chemother. 58: 101–107], a significant decrease in resistance was not expected in 2010. In 2010, two ceftiofur resistant *E. coli* were isolated from pigs. In 2009–2010, a supplementary investigation of cephalosporin resistant *E. coli* from pigs was performed by use of a selective enrichment method (Textbox 7).

Over the past decade, an overall increase in the proportion of resistant indicator *E. coli* isolates from pigs has been observed for tetracycline and ampicillin resistance, except for an unexplained peak in 2004. In the same period, use of tetracycline increased dramatically from 2002–2007 and again importantly in 2009 and first half of 2010.

In cattle, the overall occurrence of resistance to tetracycline increased significantly from 2% in 2009 to 9% in 2010 (Table 7.3, Figure 7.1), and in calves the consumption of tetracyclines increased by 10% (Table AP1.2). From 2005 to 2010, the tetracycline consumption in calves increased by 7% (absolute numbers), from 28% of the total calves consumption in 2005 to 30% in 2010; in between, a temporary decrease in the tetracycline consumption was observed concurrent with a decreasing trend in resistance. The steep increase in tetracycline resistance in 2010 might indicate a low level presence in farms that have temporarily stopped using tetracycline during 2006–2009.

7.2.2 Indicator *Escherichia coli* from meat

For Danish meat, *E. coli* were isolated from 37% (68/184) of the pork samples, 27% (32/118) of the beef samples, and 84% (158/187) of the broiler samples. For imported meat, *E. coli* were isolated from 29% (50/175) of the pork samples, 39% (39/99) of the beef samples and 78% (177/226) of the broiler samples. Only one isolate per sample was susceptibility tested and reported (Table 7.3).

MIC distributions and occurrence of antimicrobial resistance in *E. coli* isolates collected from broiler meat, pork and beef sampled at wholesale and retail outlets in 2010 are presented in Appendix 1 (Table AP1.23).

Among the indicator *E. coli* isolates from Danish and imported broiler meat that were susceptibility tested, 58% and 19% were fully sensitive, whereas 7% and 60% were found to be multi-resistant, respectively. For indicator *E. coli* from Danish broiler meat, the observed resistance to sulfonamide increased significantly from 8% in 2009 to 15% in 2010. As for indicator *E. coli* from broilers, the level of resistance in isolates from broiler meat was very low-moderate for all tested agents, with the highest level found for ampicillin (16%) (Table 7.3).

In imported broiler meat, the level of resistance was significantly higher for 13 of the 16 tested antimicrobial agents when compared to *E. coli* from Danish broiler meat, including tetracycline, chloramphenicol, ampicillin, cefoxitime, ceftiofur, colistin, sulfonamide, trimethoprim, neomycin, spectinomycin, streptomycin, ciprofloxacin and nalidixic acid (Table 7.3).

In 2010, ceftiofur resistance was observed for the first time in one isolate from Danish broiler meat obtained without selective enrichment (1%); however, this is significantly lower than among *E. coli* from imported broiler meat (7%). The occurrence of fluoroquinolone resistance in imported broiler meat (41%) was tenfold higher than in Danish broiler meat (4%) in 2010 (as in 2009) (Table 7.3).

In imported pork, two isolates with MIC for nalidixic acid <32 µg/ml and for ciprofloxacin >0.06 µg/ml were found. One of the isolates contained transferable fluoroquinolone resistance encoded by *qnrS*.

Among the susceptibility tested indicator *E. coli* isolates from Danish and imported pork, 50% and 36% were fully sensitive, whereas 24% and 38% were found to be multi-resistant, respectively. In *E. coli* from Danish pork, sulfonamide resistance decreased significantly from 38% to 19%. In 2010, significantly lower resistance to tetracycline and sulfonamide was found in isolates from Danish pork compared to imported pork. Fluoroquinolone resistance remained low (one isolate) in Danish pork, probably due to the low fluoroquinolone consumption in Danish pigs since 2002. In imported pork, 4% of the *E. coli* isolates were resistant to fluoroquinolone. Resistance to ceftiofur was found in one isolate from Danish pork in 2010, as in the two previous years. In 2009–2010, a supplementary investigation of cephalosporin resistant *E. coli* from meat was performed by use of a selective enrichment method (Textbox 7).

The occurrence of resistance in *E. coli* isolates obtained from Danish and imported beef remained low and at comparable levels. Among the susceptibility tested indicator *E. coli* isolates from Danish and imported beef, 94% and 87% were fully sensitive, whereas 6% and 5% were found to be multi-resistant, respectively. No significant changes were observed from 2009 to 2010.

7.2.3 Comparison of resistance in *Escherichia coli* from production animals and meat

Data on the occurrence of resistance in *E. coli* isolates from animals and Danish meat are presented in Table 7.3. For most of the tested antimicrobial agents, the level of resistance in Danish meat reflected the level of resistance in the corresponding animal species with some exceptions.

The occurrence of tetracycline and sulfonamide resistance among *E. coli* from Danish pork was significantly lower than what was found among Danish pigs. The difference in the level of resistance between pork and pigs might be caused by cross contamination at the slaughterhouse or due to some resistant isolates being less fit to survive through the food production chain than susceptible isolates.

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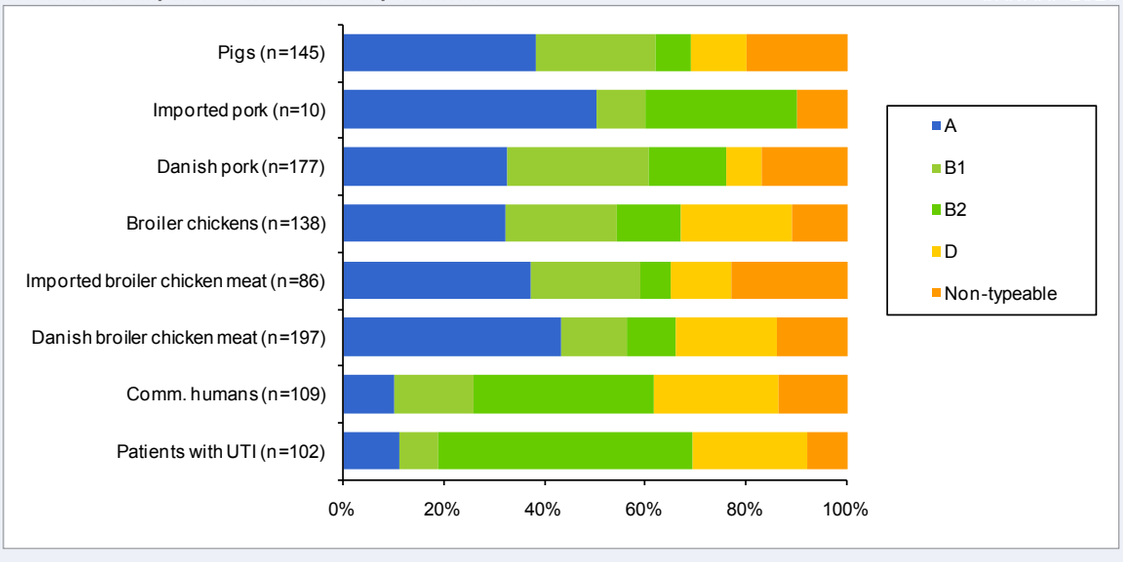
Zoonotic aspects of *E. coli* urinary tract infections

Background: Urinary tract infection (UTI) is one of the most common bacterial infections. The major aetiological agent of UTI is *Escherichia coli* accounting for 80-90% of all infections. Extraintestinal pathogenic *E. coli* (ExPEC) are characterized by carrying a number of virulence genes that enable the isolates to cause infection outside the intestinal tract, e.g. by adhering to host surfaces, scavenge iron (or other micronutrients), or evade host response. Furthermore, resistance to antibiotics in ExPEC is increasing which complicates treatment. Especially, ExPEC belonging to phylogroup B2 and to a lesser extent D cause the majority of UTI. Likewise, clonal groups and epidemic strains causing UTI have been identified. The *E. coli* causing UTI is believed to stem from the patient's own faecal flora. However, the exterior reservoir of such *E. coli* in the human intestines is poorly investigated. The food supply may transmit ExPEC from animals to humans. Since the start of DANMAP in 1995, *E. coli* from food animals at slaughter and fresh retail meat have been collected. Furthermore, *E. coli* isolates have been collected from healthy humans from 2002 through 2006. DANMAP isolates (from 2004) have recently been compared with *E. coli* isolates from patients with UTI to test the hypothesis if UTI is a zoonosis.

Materials and methods: A total of 964 geographically and temporally matched *E. coli* isolates from UTI patients, community-dwelling humans, Danish and imported broiler chicken meat, healthy Danish broiler chickens, Danish and imported pork and healthy Danish pigs were included. All isolates were investigated for phenotypic antimicrobial resistance, for phylogroups (A, B1, B2, D) and for eight ExPEC-related virulence genes (*kpsM* II, *papA* and *papC*, *iutA*, *sfaS*, *focG*, *afa* and *hlyD*) [Jakobsen *et al.* 2010. Foodborne Pathog Dis. 7: 537–47; Jakobsen *et al.* 2010. Int J Food Microbiol. 142: 264–72]. Despite many studies investigating this hypothesis using molecular characterization, *in vivo* studies have been lacking. Therefore, a subset of B2 *E. coli* isolates (n = 13) (type 1-fimbriaeted and positive for two or more of the virulence genes *iutA, kpsM II, papA, papC, focG, sfaS,* or *hlyA*) from animals and meat were investigated for their ability to cause infection in a murine model of ascending UTI [Jakobsen *et al.* 2010. J Clin Microbiol. 48: 2978–80]. The model is representative of UTI in humans. Further, D isolates from all origins (n = 158) were screened for Clonal group A (CgA) status. This clonal group cause invasive disease in humans, mainly UTI, but the distribution and possible sources are poorly investigated. Identified CgA isolates were investigated for clonal relationship and virulence in the murine UTI model [Jakobsen *et al.* 2010. Appl Environ Microbiol. 2010 76: 8281–4].

Figure 1. Distribution (%) of phylogroups and non-typeables among *Escherichia coli* isolates from animals, meat and humans, Denmark

DANMAP 2010



Results and discussion: Phylogroup B2 and D isolates were detected in different frequencies among isolates from all origins, including food animals and retail meat (except for imported pork) (Figure 1**).** Phylogroup B2 was predominant among UTI and community-dwelling human isolates and phylogroup A was predominant among animal and meat isolates. Animal and meat isolates were similar to UTI isolates based on the phenotypic antimicrobial resistance [Jakobsen *et al.* 2010. Foodborne Pathog Dis. 7: 537–47]. Although B2 and D isolates were found in low frequencies among animal and meat isolates, they may pose a risk for acquiring potentially uropathogenic *E. coli* from these sources. The detection of seven out of eight ExPEC-related virulence genes among animal and meat isolates and clustering of animal and meat isolates with UTI isolates based on antimicrobial resistance and virulence genes supported this finding [Jakobsen *et al.* 2010. Int J Food Microbiol. 142: 264–72]. All 13 investigated B2 isolates from animal and meat isolates were virulent in the murine UTI model with bacterial counts in urine, bladder and often kidneys (n = 9) providing solid evidence that UTI is at times a zoonosis [Jakobsen *et al.* 2010. J Clin Microbiol. 48: 2978–80]. This was further supported by the finding of 25 CgA isolates among isolates from broiler chickens and meat, community-dwelling humans and UTI patients. Although no CgA animal or meat isolates were clonally related to any human isolate, several community-dwelling isolates were related to UTI isolates despite no known relation. Retail meat may have been a common source. Like the B2 isolates, the CgA phylogroup D animal and meat isolates were virulent in the murine bladder and murine kidneys [Jakobsen *et al.* 2010. Appl Environ Microbiol. 76: 8281–4].

Conclusion: Food animals and fresh retail meat are sources of B2 and D isolates including invasive human Clonal group A isolates. Meat and animal isolates are similar to human isolates with respect to resistance and virulence genes. Further, animal and meat isolates can cause infection in bladder and kidneys in a murine model providing evidence that UTI can be a zoonosis.

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Occurrence of Extended spectrum beta-lactamase (ESBL)-producing *Escherichia coli* after selective enrichment with ceftriaxone in meat and food producing animals

Background: Extended spectrum beta-lactamase (ESBL)-producing bacteria is one of the fastest emerging resistance problems worldwide. The resistance type seems to be related to food producing animals and may spread to humans via food. June 2010, the use of cephalosporins in the Danish pig production was discontinued, but it is still used for systemic and intramammary treatment in cattle. Cephalosporins have not been used in the Danish broiler production for at least a decade. The aim of this study was to investigate the occurrence of ESBL-producing *E. coli* in pigs at farm level, in cattle and broilers at slaughter and in meat at retail (see Appendix 2 for definition of ESBL).

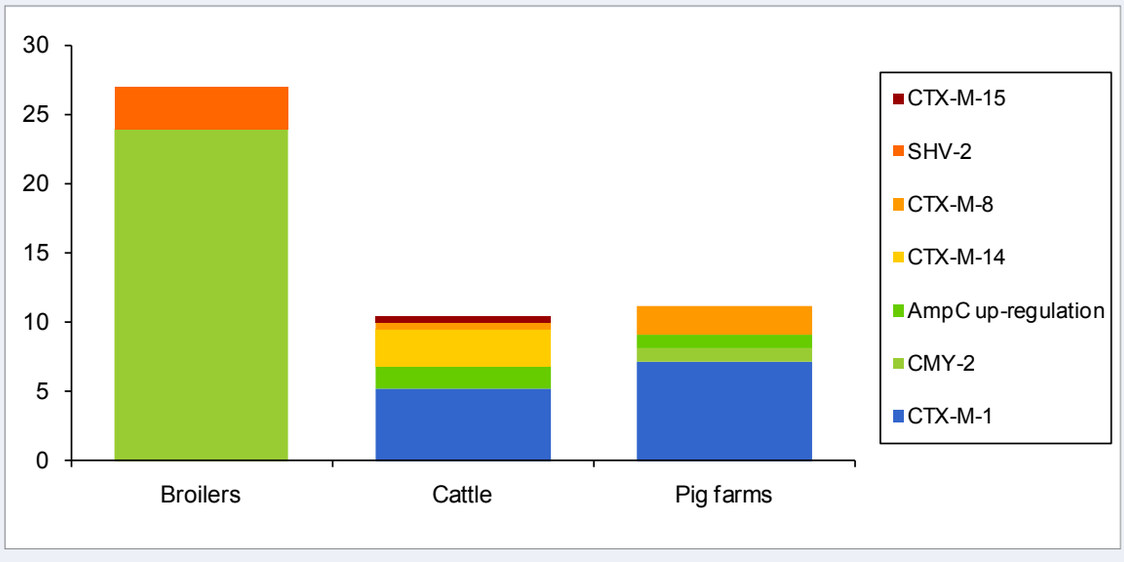
Materials and methods: During June 2010 through December 2010, stool samples (n = 99) were collected in pig farms, fecal samples (n = 192) were taken from cattle at slaughter and pools of five cloacal swabs (n = 197) were taken from broiler flocks at slaughter. From January through November 2010, 990 meat samples [Danish: Pork (n = 184), broiler meat (n = 187), and beef (n = 118); imported: Pork (n = 176), broiler meat (n = 226), and beef (n = 99)] were collected in retail stores and outlets. The samples were randomly selected and for pigs each farm was sampled only once. For cattle, one animal represented one farm. For broilers, five animals per flock were included in the pooled sample. No broiler flock or cattle herd was sampled more than once in the same month.

The meat samples were collected randomly in all regions of Denmark. *E. coli* was isolated from 1 g of pig stool sample, 1 g of cattle feces, pools of five cloacal swabs or 5 g of meat after selective enrichment in McConkey media with ceftriaxone (1 µg/ml). The genetic background for ESBL resistance was revealed by use of PCR, array and DNA sequencing.

Results: Eleven percent (11/99) of the pig stool samples contained ceftriaxone resistant *E. coli* (ESBL-producing including up-regulation of chromosomal AmpC). Among these isolates, eight (64%) contained CTX-M-1, two (18%) contained CTX-M-2, one isolate contained CMY-2 (9%) and one was AmpC up-regulated (9%). For cattle, 10% (20/192) of the faecal samples contained ceftriaxone resistant *E. coli*. Among these isolates, ten contained CTX-M-1 (50%), five contained CTX-M-14 (25%), one contained CTX-M-15 (5%), one contained CTX-M-8 (5%) and three were AmpC up-regulated (15%). For broilers, 27% (53/197) contained ceftriaxone resistant *E. coli* and these isolates contained CMY-2 (89%) and SHV-2 (11%) (Figure 1).

Figure 1. Occurrence (%) of ESBL-genes and AmpC up-regulated *Escherichia coli* in broilers, cattle and pig farms, Denmark

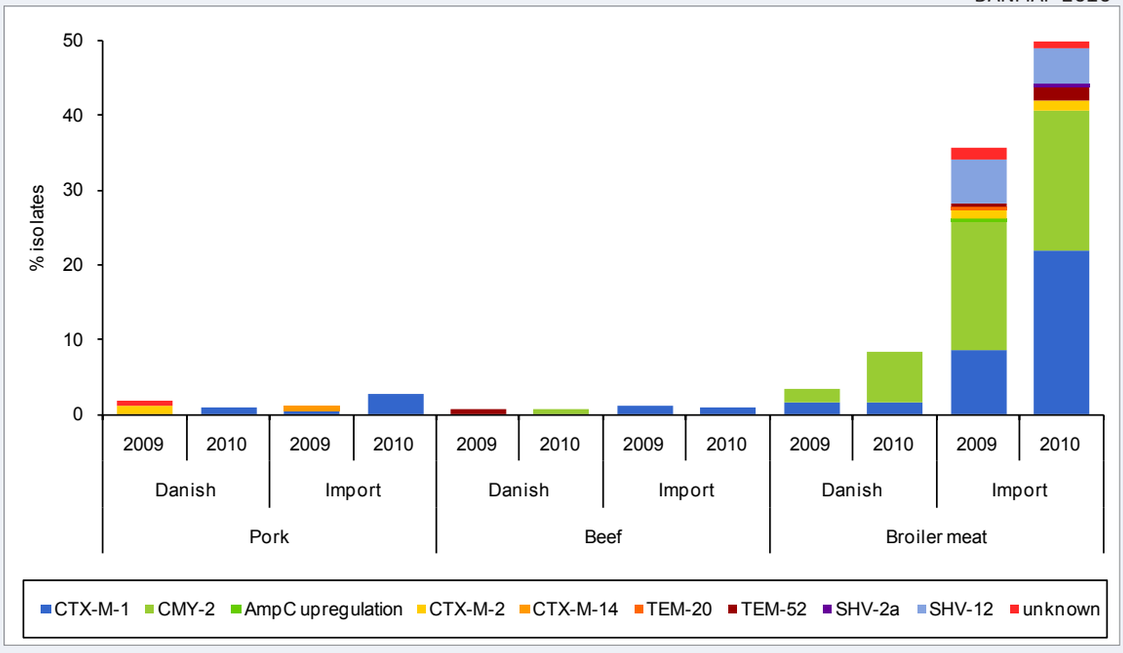
DANMAP 2010



From meat samples, the highest prevalence of ceftriaxone resistant *E. coli* was found among imported poultry (50%). Ceftriaxone resistant *E. coli* were found in 8.6% of isolates from Danish broiler meat and in 0.8 - 2.8% of isolates from the other meat catagories. Among the 113 ceftriaxone resistant *E. coli* from imported broiler meat, 42 contained CMY-2 (37%), 50 contained CTX-M-1 (44%), 11 contained SHV-12 (10%), three contained CTX-M-2 (3%), five had other mechanisms (4%) (SHV-2a, TEM-52) and two (2%) had unknown mechanisms. Among the other meat categories, CMY-2 and CTX-M-1 were found (Figure 2).

Figure 2. Occurence (%) of ESBL-genes and AmpC up-regulated *Escherichia coli* in meat, Denmark

DANMAP 2010



Discussion and conclusions: The use of selective enrichment with ceftriaxone revealed ESBL-producing *E. coli* in food producing animals, which were not found by standard monitoring of indicator *E. coli* as previously described [DANMAP 2009]. The highest prevalence of ESBL was found in broilers; this was surprising since cephalosporins are not used in the Danish broiler production. The occurrence of ESBL-producing *E. coli* found in pigs in 2010 corresponded to the occurrence reported for pigs in 2009 [DANMAP 2009]. The occurrence of ESBL in cattle at slaughter in 2010 was similar to the occurrence of ESBL producing *E. coli* in pigs.

The presence of ESBL-genes differed depending on animal reservoir. CMY-2 and SHV-2 seemed to be more related to the broiler production, whereas CTX-M-8 was found only in cattle. Even though ESBL-producing *E. coli* are present in Danish pig farms and in cattle at slaughter, the most important meat source seemed to be imported poultry. The presence of ESBL-producing *E. coli* in imported broiler meat has increased significantly from 36% in 2009 to 50% in 2010, mainly due to a higher prevalence of CTX-M-1. The occurrence of ESBL-producing *E. coli* in the other meat categories was at the same levels as in 2009.

Although it seems like the occurrence of ESBL-producing *E. coli* in Danish broiler meat was higher in 2010 than in 2009, no statistically significant difference ($p = 0.07$) was observed. Since broilers and broiler meat seem to be an important reservoir for ESBL-producing *E. coli,* also in countries like Denmark with no consumption of cephalosporins in the broiler production, more effort should be done to investigate factors important for selection of ESBL in the broiler production.

Several of the ESBL-genes detected among *E. coli* obtained from animals and meat can also be detected in *E. coli* of human origin; CTX-M-15 is most often detected in ESBL-producing *E. coli* from both urine and bloodstream infections in Danish patients, whereas CTX-M-1, CTX-M-14 and CMY-2 are present to lesser extent [Leihof *et al.* 2011. ECCMID Poster 660; Hansen *et al.* 2011. ECCMID Poster 662]. CTX-M-14 and CMY-2 have been detected in *E. coli* obtained from faecal samples from healthy Danish military recruits, indicating a human faecal reservoir of ESBL-genes in the community [Hammerum *et al.* 2011. Clin Microbiol Infect. 17: 566–8]. More studies are needed to investigate the possible transfer of ESBL-producing *E. coli* or ESBL-genes between humans and animals.

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8. Resistance in human clinical bacteria

8.1 *Escherichia coli*

Escherichia coli is part of the normal intestinal flora of both humans and animals but also cause infections (Textbox 6). In humans, *E. coli* cause a variety of intestinal and extra-intestinal infections such as diarrhoea, urinary tract infections, meningitis, and bloodstream infections. For *E. coli*, this report includes data from 14 Departments of Clinical Microbiology (DCM), representing 95% of the Danish population. Data on antimicrobial resistance in blood and urine isolates of *E. coli* in hospitals were obtained from 14 of the 15 Danish DCM working with hospital isolates; 13 DCM of the 14 DCM working with primary health care isolates contributed data on antimicrobial resistance in urine isolates of *E. coli* from primary health care (Table 8.1).

E. coli blood isolates obtained from hospitalised patients

The antimicrobial susceptibility of approximately 3,400 *E. coli* isolates from blood was reported in 2010 (Table 8.1 and Figure 8.1).

In 2010, cefuroxime (2nd generation cephalosporin) resistance was 8% (min. 3%, max. 15%) the same as reported in 2009. Likewise, 7% (min. 4%, max. 13%) of the isolates were resistant to 3rd generation cephalosporin (reported as ceftazidime, ceftriaxone, cefpodoxime or cefotaxime) similar to the level in 2009. Third generation cephalosporin resistance can be due to production of ESBL or AmpC enzymes. The genetic background was not reported for the 3rd generation cephalosporin resistant *E. coli* isolates in 2010. A study of *E. coli* from bloodstream infections obtained from three DCM in 2009 has shown that ESBL-producing enzymes are the most frequent cause of resistance to 3rd generation cephalosporins, with CTX-M-15 being the most frequent enzyme. In 2009, CTX-M-15 was spread due to both clonal and non-clonal strains [Hansen *et al.* 2011 ECCMID poster 660].

In 2010, ciprofloxacin resistance was 14% (min. 7%, max. 22% at the individual DCM) and nalidixic acid resistance was 18% (min. 8%, max. 21%), which is the same level as in 2009.

Table 8.1. Resistance (%) in *Escherichia coli* isolates from humans, Denmark 2010 DANMAP 2010

Antimicrobial agent	Blood isolates, hospitals[a]	Urine isolates, hospitals[b]	Urine isolates, primary health care[c]
	%	%	%
Ampicillin	46	41 #	40 #
Mecillinam	9 *	7	6
Sulfonamide		35 #	37 #
Gentamicin	6 *	4	3
Ciprofloxacin	14	12 #	11
Nalidixic acid	18	15	15 *
Cefuroxime	8	5 #	3
3rd generation cephalosporins [d]	7	5	3
Meropenem	0		
Max. number of isolates tested	3426	36149	29421

*) An asterisk indicates a significant increase from 2009 to 2010

#) A number sign indicates a significant decrease from 2009 to 2010

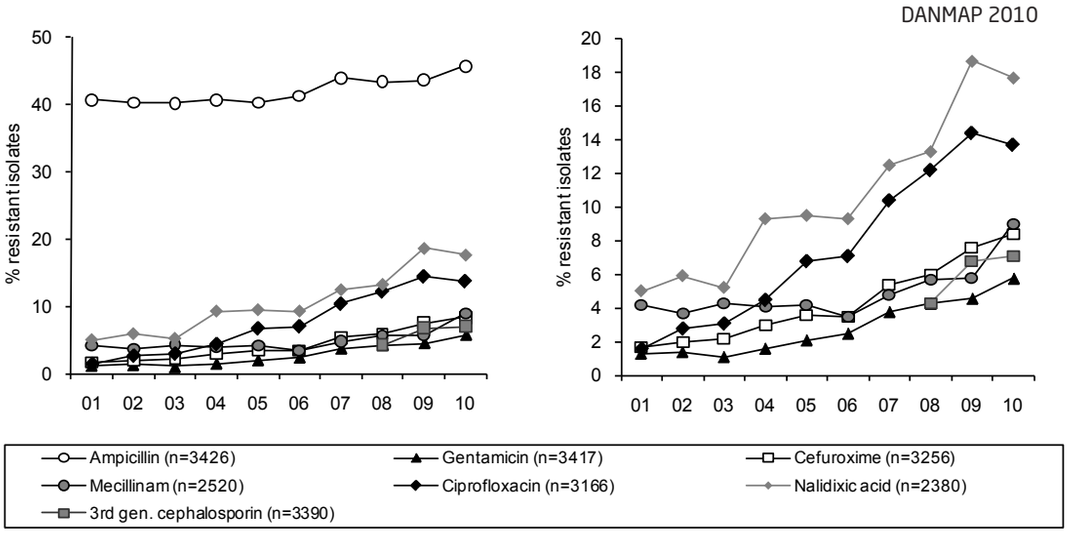
a) 14 DCM reported data on ampicillin, gentamicin and 3rd generation cephalosporin resistance, 13 DCM reported ciprofloxacin and cefuroxime resistance, 10 DCM reported mecillinam and nalidixic acid resistance, and 8 DCM reported data on meropenem resistance. Data on sulfonamide resistance were not reported

b) 14 DCM reported data on ampicillin and mecillinam resistance, 12 DCM reported cefuroxime resistance, 10 DCM reported gentamicin and 3rd generation cephalosporin resistance, and 9 DCM reported data on sulfonamide, ciprofloxacin and nalidixic acid resistance. No comparison of gentamicin resistance in 2009 and 2010 was made, since data were not reported in 2009. Since resistance to meropenem was only reported from one DCM, data are not shown

c) 13 DCM reported data on ampicillin and mecillinam resistance, 11 DCM reported sulfonamide resistance, 9 DCM reported nalidixic acid and 3rd generation cephalosporin resistance, 8 DCM reported ciprofloxacin resistance, and 7 DCM reported data on gentamicin and cefuroxime resistance. No comparison of gentamicin resistance in 2009 and 2010 was made, since data were not reported in 2009

d) Tested 3rd generation cephalosporins were ceftazidime, ceftriaxone, cefpodoxime and cefotaxime

Figure 8.1. Resistance (%) in *Escherichia coli* blood isolates from humans, Denmark



Note: The number (n) in parentheses represents the number of isolates tested for susceptibility in 2010

The level of fluoroquinolone and 3rd generation cephalosporin resistance in Denmark was again above the level reported to EARS-Net by the other Nordic countries and corresponded to the occurrence reported by other European countries in 2009 [EARS-Net 2009].

A small but significant increase in aminoglycoside (gentamicin) resistance was observed from 5% in 2009 to 6% in 2010. This corresponded to the level reported to EARS-Net by the other Nordic countries in 2009 [EARS-NET 2009]. Among the 10 DCM reporting data in both 2009 and 2010, mecillinam resistance increased significantly from 5% to 9% (in 2010, min. 2%, max. 17%).

Over the last decade, resistance to cefuroxime has increased from 2% in 2001 to 8% in 2010. Resistance to 3rd generation cephalosporins has only been reported since 2008. Resistance to fluoroquinolones has also increased over the last ten years; ciprofloxacin resistance increased from 2% in 2001 to 14% in 2010, and nalidixic acid resistance from 5% in 2001 to 18% in 2010. Aminoglycoside (gentamicin) resistance has increased from 1% in 2001 to 6% in 2010.

In 2010, carbapenem (meropenem) resistance was not observed in *E. coli* blood isolates.

E. coli urine isolates obtained from hospitalised patients

The antimicrobial susceptibility of approximately 36,000 *E. coli* isolates obtained from hospitalised patients with a urinary tract infection was reported in 2010 (Table 8.1 and Figure 8.2).

From 2009 to 2010, smaller but significant decreases in resistance were observed for the following antimicrobial agents: ampicillin (42% in 2009, 41% in 2010), sulfonamide (36% in 2009, 35% in 2010), ciprofloxacin (13% in 2009, 12% in 2010) and cefuroxime (2nd generation cephalosporin) (6% in 2009, 5% in 2010). However, over the last decade the occurrence of resistance to fluoroquinolones has increased; ciprofloxacin resistance has increased from 1% in 2001 to 12% in 2010, and nalidixic acid resistance from 5% in 2001 to 15% in 2010.

In 2010, carbapenem (meropenem) resistance was observed in two *E. coli* urine isolates from hospitalised patients. The carbapenem resistant isolates were not further investigated. The presence of antimicrobial resistance in this species is not mandatory reportable, and no calculation of the occurrence of carbapenem resistance could be made since only one DCM reported data on all isolates.

E. coli urine isolates obtained from primary health care

The antimicrobial susceptibility of approximately 29,000 *E. coli* isolates obtained from patients with a urinary tract infection from primary health care was reported in 2010 (Table 8.1 and Figure 8.3).

A small but significant increase in nalidixic acid resistance was observed from 14% in 2009 to 15% in 2010. Also, over the last decade the occurrence of resistance to fluoroquinolones has increased; ciprofloxacin resistance from 1% in 2001 to 11% in 2010, and nalidixic acid resistance from 5% in 2001 to 15% in 2010, respectively.

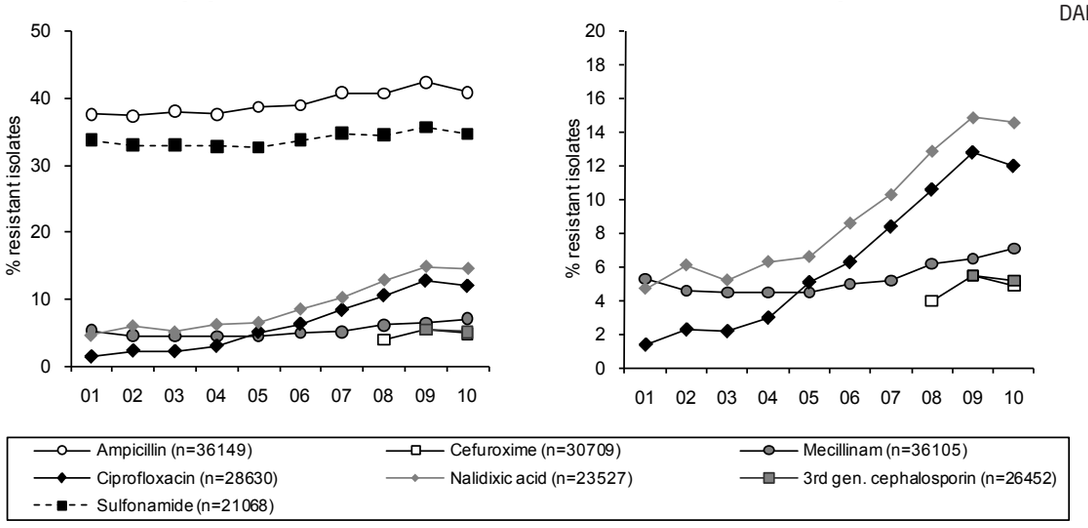
From 2009 to 2010, smaller but significant decreases in resistance were observed for ampicillin (42% in 2009, 40% in 2010) and sulfonamide (38% in 2009, 37% in 2010).

In 2010, carbapenem (meropenem) resistance was observed in five *E. coli* urine isolates from primary health care. The carbapenem resistant isolates were not further investigated. The presence of antimicrobial resistance in this species is not mandatory reportable, and no calculation of the occurrence of carbapenem resistance could be made since the DCM reported data on selected isolates only.

Line Skjøt-Rasmussen, Stefan S. Olsen and Anette M. Hammerum

Figure 8.2. Resistance (%) in *Escherichia coli* urine isolates from humans in hospitals, Denmark

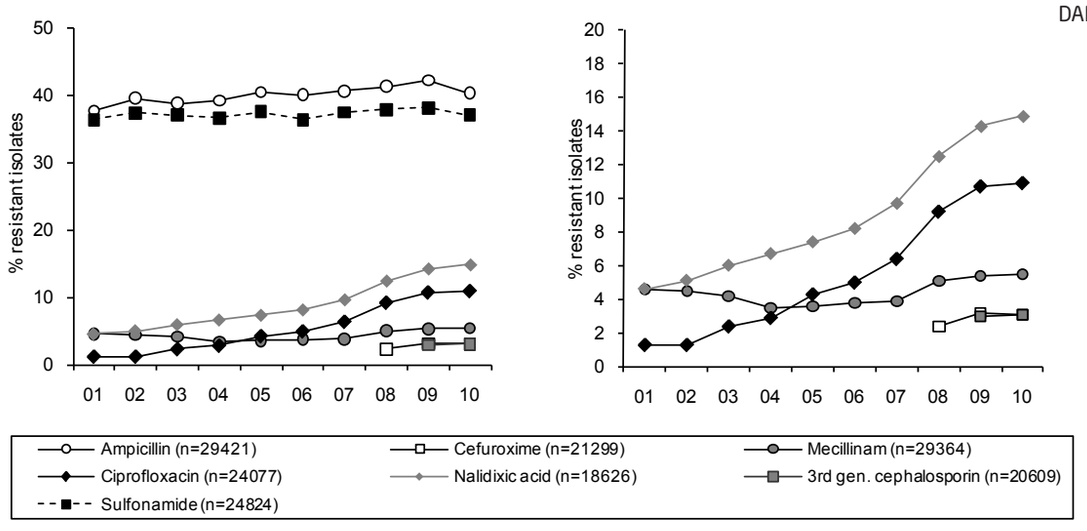
DANMAP 2010



Note: The number (n) in parentheses represents the number of isolates tested for susceptibility in 2010

Figure 8.3. Resistance (%) in *Escherichia coli* urine isolates from humans in primary health care, Denmark

DANMAP 2010



Note: The number (n) in parentheses represents the number of isolates tested for susceptibility in 2010

8.2 *Klebsiella pneumoniae*

Klebsiella pneumoniae is part of the intestinal flora in humans but is often the cause of extra-intestinal infections such as urinary tract-, respiratory tract-, wound- and bloodstream infections. Many of these infections are hospital acquired and can be life threatening, especially if the strains are resistant to antimicrobial agents. *K. pneumoniae* is intrinsically resistant to aminopenicillins (e.g. ampicillin). Therefore, infections caused by *K. pneumoniae* are treated with broad spectrum antimicrobial agents such as ciprofloxacin, gentamicin, cephalosporins and carbapenems. Data on antimicrobial resistance in blood and urine isolates of *K. pneumoniae* in hospitals were obtained from 14 of the 15 Danish DCM working with hospital isolates; 13 of the 14 DCM working with primary health care isolates contributed data on antimicrobial resistance in urine isolates of *K. pneumoniae*.

K. pneumoniae blood isolates obtained from hospitalised patients

The antimicrobial susceptibility of approximately 800 *K. pneumoniae* isolates from blood was reported in 2010 (Table 8.2).

Until 2007, the occurrence of antimicrobial resistance in *K. pneumoniae* was low and at the same level as in the other Nordic countries (e.g. for 3rd generation cephalosporins <5%). However, since 2007 a steady increase in resistance has been observed until 2009. When comparing 2010 with 2009, a significant decrease was observed for gentamicin, ciprofloxacin and cefuroxime resistance; this was mostly due to decreased occurrence of these resistances in *K. pneumoniae* isolates from Zealand. This could in part be explained by interventions at hospitals in the Copenhagen area (Textbox 8).

In 2010, 3rd generation cephalosporin resistance (reported as ceftazidime, ceftriaxone, cefpodoxime or cefotaxime) was 9% (min. 4%, max. 24%); this was at the same level as in 2009 (12%). In 2010, 3rd generation cephalosporin resistance was above the level reported to EARS-Net by the other Nordic countries and corresponded to the occurrence reported by several other European countries in 2009 [EARS-Net 2009]. In the Eastern part of Denmark (Zealand), 3rd generation cephalosporin resistance in *K. pneumoniae* (14%) was significantly higher than in the Western part (Funen and Jutland) (6%). Third generation cephalosporin resistance can be due to production of ESBL or AmpC enzymes. The genetic background was not reported for the 3rd generation cephalosporin resistant *K. pneumoniae* isolates in 2010. In a study from 2008, it was observed that 3rd generation cephalosporin resistant *K. pneumoniae* from bloodstream infections in Danish hospitals was mostly due to spread of two clones (ST15 and ST16) producing the ESBL-enzyme CTX-M-15 among hospitals in Zealand. Both clones have been reported in other countries before, indicating international spread [Lester *et al.* 2011, Int J Antimicrob Agents. 38(2): 180-2].

The occurrence of fluoroquinolone resistance (ciprofloxacin 11%, nalidixic acid 17%) and aminoglycoside (gentamicin) resistance (6%) was above the level reported from the other Nordic countries and the same as reported to EARS-Net by other European countries in 2009 [EARS-Net 2009].

In 2010, carbapenem (meropenem) resistance was not observed in *K. pneumoniae* blood isolates.

K. pneumoniae urine isolates obtained from hospitalised patients

The antimicrobial susceptibility of approximately 5,500 *K. pneumoniae* isolates obtained from hospitalised patients with a urinary tract infection was reported in 2010 (Table 8.3).

The occurrence of 3rd generation cephalosporin resistance was 12% (reported as cefpodoxime or cefotaxime) and corresponded to the occurrence reported in 2009 (13%). In the Eastern part of Denmark (Zealand), 3rd generation cephalosporin resistance in *K. pneumoniae* (20%) was significantly higher than in the Western part (Jutland) (7%).

Fluoroquinolone resistance decreased from 2009 to 2010; ciprofloxacin resistance decreased from 17% to 14%, and nalidixic acid resistance decreased from 22% to 20%. In the Eastern part of Denmark (Zealand), the occurrence of ciprofloxacin resistance (16%) was significantly higher than in the western part (Jutland and Funen) (7%).

In 2010, carbapenem (meropenem) resistance was observed in seven *K. pneumoniae* urine isolates from hospitalised patients. The seven isolates were tested for the presence of carbapenem resistance genes. One of the seven isolates produced the new carbapenemase enzyme New Delhi metallo-β-lactamase 1 (NDM-1) and was resistant towards all tested antimicrobial agents except tigecycline and colistin. This isolate was obtained from urine from a colonised patient [Hammerum *et al.* 2010, Lancet Infect Dis 10: 829-30]. The patient had been travelling to Bosnia and Herzegovina; a Balkan link has also been found in other countries with the

Table 8.2. Resistance (%) in *Klebsiella pneumoniae* isolates from blood, Denmark

DANMAP 2010

Antimicrobial agent	2008[a] %	2009[b] %	2010[c] %
Gentamicin	7.6	9.0	6.0
Ciprofloxacin	16.1	18.1	11.3
Nalidixic acid	22.2	21.8	17.4
Cefuroxime	15.2	17.1	12.8
3rd gen. cephalosporins [d]	9.6	12.0	9.2
Meropenem	0.2	0	0
Max. number of isolates tested	788	886	799

a) 14 DCM reported data on gentamicin resistance, 13 DCM reported ciprofloxacin and cefuroxime resistance, 11 DCM reported 3rd gen. cephalosporin resistance, 10 DCM reported nalidixic acid resistance, and 9 DCM reported data on meropenem resistance
b) 14 DCM reported data on ciprofloxacin and gentamicin resistance, 13 DCM reported cefuroxime resistance, 12 DCM reported 3rd gen. cephalosporin resistance, 10 DCM reported nalidixic acid resistance and 9 DCM reported data on meropenem resistance
c) 14 DCM reported data on gentamicin and 3rd gen. cephalosporin resistance, 13 DCM reported cefuroxime resistance, 11 DCM reported ciprofloxacin resistance, 10 DCM reported nalidixic acid resistance and 8 DCM reported data on meropenem resistance
d) Tested 3rd generation cephalosporins were ceftazidime, ceftriaxone, cefpodoxime and cefotaxime

Table 8.3. Resistance (%) in *Klebsiella pneumoniae* urine isolates from humans in hospitals, Denmark

DANMAP 2010

Antimicrobial agent	2009[a] %	2010[b] %
Mecillinam	13.3	14.3
Sulfonamide	27.0	28.6
Gentamicin		7.3
Ciprofloxacin	17.3	13.9
Nalidixic acid	21.9	19.6
Cefuroxime		12.8
3rd gen. cephalosporins [c]	12.8	12.0
Max. number of isolates tested	6394	5740

a) 12 DCM reported data on mecillinam, sulfonamide and ciprofloxacin resistance, 8 DCM reported nalidixic acid and 3rd generation cephalosporin resistance, and 1 DCM reported data on meropenem resistance (not shown). Gentamicin and cefuroxime resistance was not reported
b) 14 DCM reported data on mecillinam resistance, 12 DCM reported cefuroxime resistance, 10 DCM reported gentamicin and 3rd generation cephalosporin resistance, 9 DCM reported sulfonamide, ciprofloxacin and nalidixic acid resistance, and 1 DCM reported data on meropenem resistance (not shown)
c) Tested 3rd generation cephalosporins were cefpodoxime and cefotaxime

first NDM-1 isolates [Struelens *et al.* 2010, Euro Surveill. 15 pii: 19716]. Another of the seven isolates was positive for VIM-1, whereas the remaining five carbapenem resistant isolates were not positive for any of the known carbapenem resistance genes. The presence of antimicrobial resistance in this species is not mandatory reportable, and no calculation of the occurrence of carbapenem resistance could be made since only one DCM reported data on all isolates.

Sulfonamide resistance increased significantly from 27% in 2009 to 29% in 2010.

K. pneumoniae urine isolates obtained from primary health care

The antimicrobial susceptibility of approximately 3,000 *K. pneumoniae* isolates obtained from patients with a urinary tract infection from primary health care was reported in 2010 (Table 8.4).

The occurrence of 3rd generation cephalosporin resistance was 7% (reported as cefpodoxime or cefotaxime) and corresponded to the occurrence reported in 2009 (8%). In the Eastern part of Denmark (Zealand), 3rd generation cephalosporin resistance in *K. pneumoniae* (11%) was significantly higher than in the Western part (Jutland) (4%). Resistance to 3rd generation cephalosporins in *K. pneumoniae* from urine from general practice patients was significantly lower than the occurrence of resistance detected in isolates from both blood and urine from hospitalised patients.

Table 8.4. Resistance (%) in *Klebsiella pneumoniae* urine isolates from humans in primary health care, Denmark DANMAP 2010

Antimicrobial agent	2009[a] %	2010[b] %
Mecillinam	15.1	16.3
Sulfonamide	30.2	33.9
Gentamicin		3.5
Ciprofloxacin	13.2	11.9
Nalidixic acid	19.9	19.7
Cefuroxime		8.6
3rd gen. cephalosporins [c]	8.1	7.0
Max. number of isolates tested	3200	3200

a) 11 DCM reported data on mecillinam and sulfonamide resistance, 9 DCM reported ciprofloxacin resistance, and 7 DCM reported nalidixic acid and 3rd generation cephalosporin resistance. Meropenem resistance was only reported for selected isolates and therefore not shown. Gentamicin and cefuroxime resistance was not reported

b) 13 DCM reported data on mecillinam resistance, 11 DCM reported sulfonamide resistance, 9 DCM reported nalidixic acid and 3rd generation cephalosporin resistance, 8 DCM reported ciprofloxacin resistance, and 7 DCM reported gentamicin and cefuroxime resistance. Meropenem resistance was only reported for selected isolates and therefore not shown

c) Tested 3rd generation cephalosporins were cefpodoxime and cefotaxime

Fluoroquinolone resistance was 20% for nalidixic acid and 12% for ciprofloxacin, corresponding to the occurrences observed in 2009. However, in the Eastern part of Denmark (Zealand), the occurrence of ciprofloxacin resistance (21%) was significantly higher than in the western part (Jutland and Funen) (7%). Ciprofloxacin resistance in *K. pneumoniae* from urine from general practice patients was significantly lower than the occurrence of resistance detected in isolates from urine from hospitalised patients.

In 2010, carbapenem (meropenem) resistance was observed in one *K. pneumoniae* urine isolate from primary health care. The carbapenem resistant isolate was not further investigated. The presence of antimicrobial resistance in this species is not mandatory reportable, and no calculation of the occurrence of carbapenem resistance could be made since the DCM reported data on selected isolates only.

Sulfonamide resistance increased significantly from 30% in 2009 to 34% in 2010. Resistance to mecillinam was 16% (min. 7%, max. 24%). Both resistance to sulfonamide and mecillinam was significantly higher in the urine isolates from general practice patients than in the urine isolates from hospitalised patients, probably reflecting the usage of sulfonamide and mecillinam in the treatment of urinary tract infections in primary health care.

Anette M. Hammerum, Stefan S. Olsen and Line Skjøt-Rasmussen

Reduction in the prevalence of ESBL-producing *Klebsiella pneumoniae* after changing the antibiotic policy and antimicrobial consumption at Bispebjerg Hospital

In 2008, an increasing prevalence of ESBL-producing *Klebsiella pneumoniae* and *E. coli* was observed in the Copenhagen City area. Especially, ESBL-producing *Klebsiella pneumoniae* was increasing in two hospitals. At the end of 2009, more than 40% of the *K. pneumoniae* isolates were ESBL-producing at these two hospitals. This increased level was seen in spite of numerous infection control initiatives such as reintroducing chlorine cleaning and focusing on isolation precautions.

An intervention was prepared for the 600 bed University Hospital, Bispebjerg Hospital, where the usage of cephalosporins was restricted to surgical prophylaxis and to the empirical treatment of meningitis, and the usage of quinolones was to be decreased significantly. In Bispebjerg Hospital and the other hospitals in the area served by the DCM Hvidovre Hospital, the use of penicillins was preferred in all cases possible, e.g. *Staphylococcus aureus* was treated with dicloxacillin, and the use of quinolones avoided. The intervention was a multidisciplinary exercise planned by a clinical microbiologist and a clinical pharmacologist and carried out in collaboration with the infections control team and the quality organisations in the hospital. Focus was set on diagnostic initiatives, isolation precautions and use of small spectrum antimicrobial agents when possible. Numerous teaching lectures were given, and written information and guidelines were distributed to all clinical working employees in brochures and electronically.

The rate of patients at Bispebjerg Hospital with ESBL-producing *K. pneumoniae* decreased from 43% in January 2010 to 16% in November 2010 ($p = 0.007$), and the rate of patients with ESBL-producing *E. coli* was unchanged at app. 12%. The number of bed-days with patients under isolation precautions for patients with ESBL-producing *K. pneumoniae* and *E. coli* was reduced from more than 260 per month to less than 50 ($p < 0.001$). The compliance to the new guidelines was almost complete; the consumption of cephalosporins decreased by 76% from 2,410 DDD/month in 2009 to 581 in 2010. Likewise, for fluoroquinolones a 16% reduction from 1,477 DDD/month in 2009 to 1,244 in 2010 was observed. The number of bed-days with patients under isolation precautions was also reduced significantly.

These outstanding effects of the intervention have resulted in a permanent change in the routine guidelines for the Bispebjerg Hospital to the above described. The effect of improved antimicrobial stewardship in the other hospital in the area with similar problems with ESBL-producing *K. pneumoniae* also resulted in a decrease in incidence of patients with ESBL-producing *K. pneumoniae.*

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Table 8.5. Resistance (%) in *Pseudomonas aeruginosa* blood isolates from humans, Denmark DANMAP 2010

Antimicrobial agent	2007 %	2008 %	2009 %	2010 %
Ciprofloxacin	5.7	4.5	5.3	5.8
Gentamicin	1.2	<1	<1	1.3
Ceftazidime	2.4	3.4	3.6	2.8
Meropenem	2.3	<1	2.5	3.1
Piperacillin / Tazobactam	3.4	2.3	1.8	3.9
Max. number of isolates tested	417	426	440	375

8.3 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is an opportunistic pathogen of immunocompromised individuals. *P. aeruginosa* typically infects the pulmonary tract, urinary tract, burns, wounds, and also causes other bloodstream infections. It is the most frequent coloniser of medical devices (e.g. catheters). *P. aeruginosa* infection is a serious problem in patients hospitalised with cancer, cystic fibrosis and burns. The case fatality rate in these patients is high.

P. aeruginosa blood isolates obtained from hospitalised patients

For *P. aeruginosa*, this report includes data from 14 Departments of Clinical Microbiology (DCM), representing 95% of the Danish population. The antimicrobial susceptibility of approximately 375 *P. aeruginosa* isolates from blood was reported in 2010. Not all DCM tested for the same antimicrobial agents (Table 8.5). The occurrence of resistance was low for all the tested antimicrobial agents and compared to the other countries reporting to the EARS-Net among the lowest.

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8.4 Streptococci

Streptococci are part of the normal commensal flora of the mouth, skin, intestine, and upper respiratory tract of humans, but streptococci also cause infections such as otitis media, tonsillitis, bacterial pneumonia, bacteremia/sepsis, endocarditis and meningitis.

In this report, data on resistance in invasive (from blood or cerebrospinal fluid) streptococcal isolates were obtained from the Neisseria and Streptococcus Reference laboratory covering all DCM in Denmark. In Denmark, penicillins and macrolides are often used for treatment of infections caused by streptococci. All invasive non-duplicate *Streptococcus pneumoniae* and group A, B, C and G streptococci were susceptibility tested against erythromycin and penicillin.

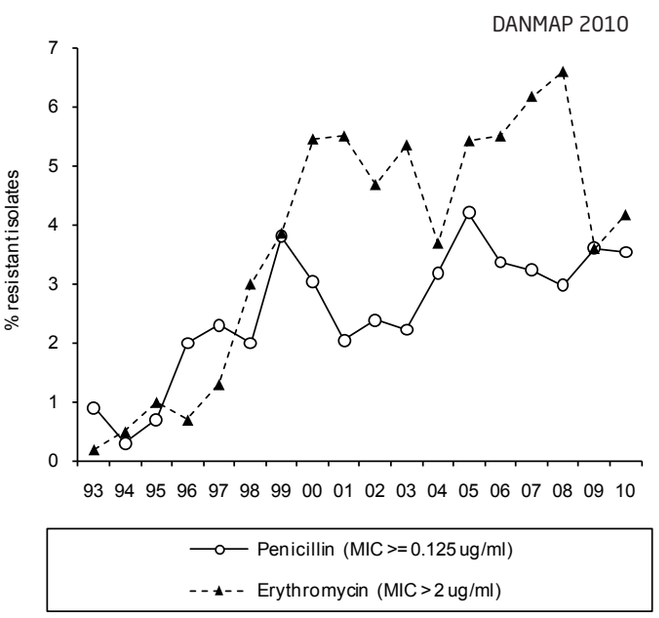
Streptococcus pneumoniae

Streptococcus pneumoniae is a leading cause of bacterial pneumonia, otitis media, bacteraemia and meningitis. In 2010, susceptibility testing was performed on 960 non-duplicate *S. pneumoniae* isolates from invasive infections (Figure 8.4).

Macrolide resistance in *S. pneumoniae* isolates from blood and cerebrospinal fluid was 4.2% (n = 40) in 2010. The occurrence of macrolide resistant *S. pneumoniae* has been around 6% from 2000 to 2008, but decreased significantly from 6.6% in 2008 to 3.6% in 2009. The decrease in the number of erythromycin resistant *S. pneumoniae* may be related to the introduction of the pneumococcal conjugated vaccine in the Danish childhood vaccination program in October 2007. The 40 macrolide resistant *S. pneumoniae* from 2010 belonged to 15 different serotypes and the most commonly found serotypes were type 19A (30%), 11A (15%) and 14 (12.5%). In previous years, serotype 14 was the dominant erythromycin resistant serotype.

As in previous years, no resistance to penicillin in group B, C or G isolates from invasive infections was reported in 2010.
The percentage of *S. pneumoniae* invasive isolates being

Figure 8.4. Resistance (%) in *Streptococcus pneumoniae* blood and spinal fluid isolates from humans, Denmark



non-susceptible (resistant and intermediary resistant) to penicillin was 3.5% (n = 34) in 2010 compared with 3.2% in 2007, 3.0% in 2008, and 3.6% in 2009. The 34 penicillin non-susceptible *S. pneumoniae* from 2010 belonged to 16 different serotypes, and the most commonly found serotypes were type 19A (38%), 15A (8.8%) and 6C (8.8%). In previous years, serotype 19A was present in a lower percentage whereas serotype 9V was a dominant type also in 2006 and 2007.

The occurrence of resistance to erythromycin and penicillin was similar to the occurrence in other Scandinavian countries but much lower than reported in many of the other European countries reporting to EARS-Net [EARS-Net 2009].

According to EUCAST, one (0.1%, MIC = 4 µg/ml) of the 960 tested isolates was resistant to penicillin (MIC > 2 µg/ml). However, according to the CLSI penicillin breakpoint for susceptibility testing of isolates from patients with invasive disease treated with intravenous penicillin, except for patients with meningitis, (2 µg/ml < MIC < 8 µg/ml) this isolate would be reported as non-susceptible intermediary resistant.

Group A Streptococci

In 2010, 155 invasive GAS (*Streptococcus pyogenes*) isolates were susceptibility tested. As in previous years, no resistance to penicillin in GAS isolates from invasive infections was reported in 2010. Erythromycin resistance was detected in two isolates (1.3%) as compared to six of 143 isolates (4.5%) in 2009 and two of 136 (1.5%) in 2008.

Group B, C and G Streptococci

In 2010, 110 invasive group B streptococci (*Streptococcus agalactiae*) isolates from invasive infections were tested. Erythromycin resistance was detected in 14 isolates (12.7%) compared with 12.8% in 2009, and 11.4% in 2008.

Fifty-three isolates of invasive group C streptococci were tested in 2010. One isolate (1.9%) was resistant to erythromycin compared with three isolates (8.1%) in 2009 and 4% in 2008.

Seventeen (12%) of the tested 142 invasive group G streptococci were resistant to erythromycin compared with 4.8% in 2009, 10% in 2008, and 8% in 2007.

Lotte M. Lambertsen

8.5 Enterococci

Enterococci are part of the normal intestinal flora of both humans and animals but can also cause infections. Important clinical infections caused by *Enterococcus* species include urinary tract infections, bacteremia and bacterial endocarditis. *E. faecalis* and *E. faecium* can cause life-threatening infections in humans, especially in the hospital environment. The naturally high level of antimicrobial resistance found in *E. faecalis* and *E. faecium* makes infections difficult to treat. Antimicrobial therapy for serious enterococcal infections requires the use of synergistic combinations of a cell-wall-active agent such as penicillin (ampicillin) and an aminoglycoside (gentamicin) or a glycopeptide (vancomycin).

For *E. faecalis* and *E. faecium*, data from 14 of the 15 DCM were obtained, representing 95% of the Danish population.

Enterococcus faecium and *Enterococcus faecalis* blood isolates obtained from hospitalised patients

In 2010, a maximum of 506 *E. faecium* isolates and 535 *E. faecalis* isolates from blood were tested for antimicrobial susceptibility.

Ampicillin resistant *E. faecium* increased significantly from 87% in 2009 to 92% in 2010. Treatment with fluoroquinolones, cephalosporins or carbapenems has been described as a risk factor for development of an *E. faecium* infection. An increasing consumption of these antimicrobial agents has been observed in hospitals in Denmark during the past years. The antimicrobial pressure in a hospital environment might be a reason for the increasing frequency of ampicillin resistant *E. faecium* as a cause of bloodstream infections.

Only one of the DCM (Aalborg Hospital) tested all enterococcal blood isolates for High-level gentamicin resistance (HLGR). Among the tested *E. faecalis* isolates at DCM Aalborg, 36% were HLGR, whereas 74% of the tested *E. faecium* isolates were HLGR. The occurrence of HLGR *E. faecalis* was similar to the occurrence detected in many countries reporting to EARS-Net in 2009 (including Spain, Portugal and Norway) [EARS-Net 2009].

Vancomycin resistance was detected in 1.8% of the *E. faecium* isolates (n = 9) and 0.7% of the *E. faecalis* isolates (n = 3) from bloodstream infections. During 2010, an outbreak of vancomycin resistant (*vanA*) *E. faecium* was detected at Aarhus University Hospital. This outbreak is under investigation (Brian Kristensen, personal communication).

The occurrence of vancomycin resistant *E. faecium* and vancomycin resistant *E. faecalis* was at the same level or lower compared to most other countries in Europe [EARS-Net 2009].
Since 2005, SSI has asked all the DCM to send presumable vancomycin resistant enterococcal isolates from both invasive and non-invasive infections for national surveillance on vancomycin resistant

enterococci. Besides the *vanA E. faecium* isolates from the outbreak at Aarhus University hospital, 19 *vanA E. faecium*, 7 *vanB E. faecium* and 5 *vanB E. faecalis* isolates were received during 2010.

As described above, most of the *E. faecium* isolates from bloodstream infections were resistant to ampicillin; these infections can therefore not be treated with ampicillin but will often be treated with vancomycin instead. This might in part, together with the increased number of MRSA infections, explain the increased consumption of glycopeptides (vancomycin) in hospitals, which has been observed during the last years.

Anette M. Hammerum, Stefan S. Olsen and Line Skjøt-Rasmussen

8.6 *Staphylococcus aureus*

Staphylococcus aureus is part of the normal flora from skin and mucosa in approximately 50% of humans. Some people only carry *S. aureus* intermittently whereas others carry *S. aureus* for longer time. However, *S. aureus* also cause infections ranging from superficial skin infections i.e. impetigo and boils, to invasive infections such as post operative wound infections, infections related to intravenous catheters and prosthetic devices, arthritis, bacteremia and endocarditis.

In Denmark, Methicillin Resistant *S. aureus* (MRSA) has been both laboratory and clinical notifiable since November 2006. In recent years, *S. aureus*, belonging to clonal complex 398 (CC398), has attracted special attention as this type has been closely connected to livestock animals, especially pigs, and has affected people in direct contact with pigs.

Surveillance of bacteremia

In 2010, 1,418 *S. aureus* bacteremia cases corresponding to 24.6 per 100,000 inhabitants were reported from the Departments of Clinical Microbiology (DCM) in Denmark. Twenty (1.4%) of the cases were caused by MRSA. This is at the same level as in previous years and very low compared to most of the other countries participating in EARS-Net [EARS-Net 2009]. Resistance in *S. aureus* bacteremia isolates from 2005–2010 is presented in Table 8.6.

Multi-resistance defined as resistance to at least 1, 2 or 3 other antimicrobials in addition to penicillin was demonstrated in 22%, 7% and 2% of the cases, respectively. In 2010, resistance to fusidic acid and norfloxacin increased compared with 2009. Eleven (0.8%) of the bacteremia cases belonged to CC398, the strain type associated to livestock. None of these were MRSA and any association to pig farming is not known. The corresponding numbers were ten in 2009, six in 2008 and five in 2007.

Surveillance of Methicillin Resistant *S. aureus*

In 2010, 1,097 new MRSA cases were detected (19.8 per 100,000 inhabitants). Here, a case is a patient found positive for the first time with a specific MRSA strain regardless whether the patient was infected or colonised.

This is a large increase (34%) compared with 817 in 2009 and is the highest number of cases observed in over 25 years (Figure 8.5). In 2010, five persons were found with two different MRSA strains. At the time of diagnosis, 646 (59%) of the new cases had infection, this was at the same level as in 2009 (486 cases (60%)). The proportion of bloodstream infections with MRSA was 1.4% in 2010 (see surveillance of *S. aureus* bacteremia). The incidence rate of new MRSA cases per year for each DCM in the last four years is shown in Table 8.7. The incidence varied from 30.9 per 100,000 inhabitants in the greater Copenhagen area (Greater Copenhagen is served by three DCM, and is shown as one) to 10.6 per 100,000 inhabitants in Vejle.

The total number of cases and the number of cases presenting with infection according to epidemiological classification are shown in Table 8.8. Most of the cases (77%) were acquired in Denmark. The epidemiological classification of MRSA infections 2006–2010 is shown in Figure 8.6. An increase was seen among cases classified as health-care associated, with community onset (HACO) from 49 cases in 2009 to 132 cases in 2010 (Figure 8.6). Only 16 of the HACO infections could be

Table 8.6. Resistance (%) in isolates from *Staphylococcus aureus* bacteraemia cases, Denmark

DANMAP 2010

Antimicrobial agent	2005 %	2006 %	2007 %	2008 %	2009 %	2010 %
Methicillin	1.6	1.4	0.6	1.3	1.6	1.4
Penicillin	78	80	78	77	77	75
Erythromycin	5	5	4	5	7	5
Clindamycin	4	4	3	4	6	4
Tetracycline	3	3	2	3	2	3
Fusidic acid	10	10	9	9	9	13
Rifampicin	<1	<1	<1	<1	<1	<1
Norfloxacin	3	2	1	2	2	3
Kanamycin	2	1	<1	1	1	1
Mupirocin	0	0	<1	<1	<1	<1
Number of isolates	1428	1329	1345	1344	1480	1418

Figure 8.5. Number of MRSA cases, with a three years moving average, Denmark

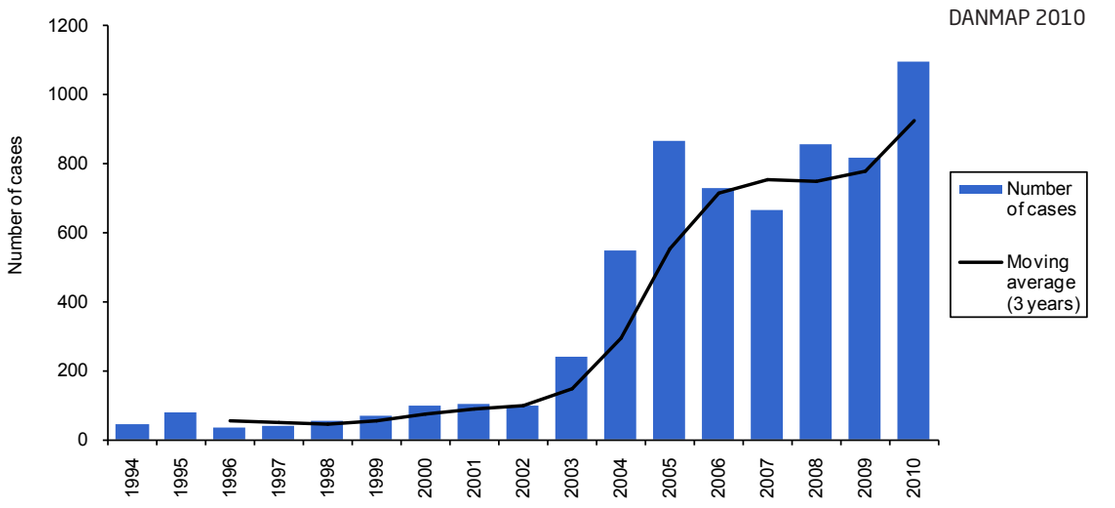


Figure 8.6. Number of MRSA cases presenting with infection according to epidemiological classification, Denmark

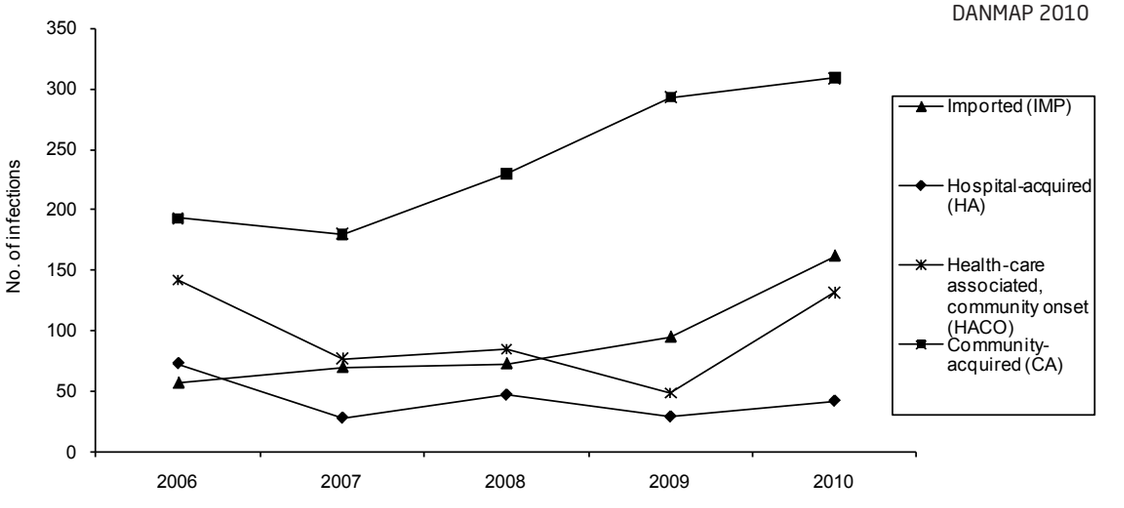


Table 8.7. Incidence rate of new MRSA cases per 100,000 inhabitants per Department of Clinical Microbiology, Denmark

DANMAP 2010

Department of Clinical Microbiology	2007	2008	2009	2010
Greater Copenhagen[a]	17.7	23.6	26.9	30.9
Hillerød	11.7	21.8	16.5	27.0
Statens Serum Institut[b]	3.9	17.6	17.6	–
Slagelse[c]	15.3	19.3	15.1	17.9
Næstved	8.8	12.1	8.8	19.8
Odense	7.1	12.9	8.9	16.3
Sønderborg	8.1	11.1	12.8	25.8
Esbjerg	14.5	8.8	6.2	11.8
Vejle	23.4	20.0	11.6	10.6
Herning	2.5	7.7	7.3	15.1
Århus	10.1	6.0	10.1	11.6
Viborg	8.2	18.5	9.1	11.0
Aalborg	8.2	10.7	7.6	16.6
Denmark total	12.1	15.5	14.7	19.8

a) Rigshospitalet (national referral hospital), Hvidovre and Herlev
b) Statens Serum Institut is no longer serving former Roskilde County
c) Including isolates from former Roskilde County

Table 8.8. Epidemiological classification of new MRSA cases, Denmark

DANMAP 2010

Epidemiologic classification	Exposure	2009		2010	
		No. of cases[a]	No. (%) of cases with infections	No. of cases[b]	No. (%) of cases with infections
Imported (IMP)		**156**	97 (62)	**247**	163 (66)
Hospital-acquired (HA)		**53**	30 (57)	**62**	34 (55)
Health-care associated, community onset (HACO)	with health care risk	**81**		**169**	
	with known exposure	**25**	12 (48)	**40**	16 (40)
	without known exposure	**56**	37 (66)	**129**	116 (90)
Health care worker		**18**	5 (28)	**35**	8 (23)
Community-acquired (CA)	without health care risk	**491**		**578**	
	with known exposure	**176**	37 (21)	**331**	100 (30)
	without known exposure	**315**	265 (84)	**247**	210 (85)
Unclassified		**4**	3 (75)	**0**	0 (0)

Note: Numbers shown in bold are totals
a) Epidemiological classification missing for 5 cases
b) Epidemiological classification missing for 6 cases

associated with a known exposition, 8 from hospitals, 3 from nursing homes and the remaining 5 from other sources. The remaining 116 cases of infection classified as HACO were registered with a possible association to health-care institutions without known exposition, of these were 62 cases with an association to hospitals, and 32 cases with an association to nursing homes and private home care. The increase in the number of HACO infections without any known exposition may reflect a better completion of the report forms where any contact to health-care institutions within the previous 12 months is registered.
Without such information, the cases would have been categorised as community-acquired (CA) infections. CA infections remained at the same level as in 2009 despite the increase in the number of infections.
In 2010, more CA cases reported known exposure compared to 2009, both for patients with infections and carriers, the latter representing contact screening.

Molecular typing of the MRSA strains

The number of isolates belonging to the 10 dominating *spa* types isolated in 2010 is shown in Table 8.9. They constituted 55% of the total number of MRSA isolates. Seven *spa* types constituted 51% of the 646 clinical infections with MRSA (out of 141 different *spa* types associated with clinical infection). Most prevalent *spa* types causing clinical infections were t002, t008, t019, t024, t034, t044 and t032. Of the 451 strains isolated from asymptomatic carriers, t034 was the most prevalent *spa* type (n = 61), followed by t002 (n = 43), t008 (n = 27), t024 (n = 27) and t127 (n = 22). The number of CC398 isolates (the clonal complex related to pigs) increased from 40 in 2009 to 109 in 2010. Among the CC398 isolates, the most frequent *spa* type, t034, increased from 27 in 2009 to 93 in 2010. Thirty-two of the 93 t034 cases represented infections (Table 8.9).

Table 8.9. The ten most prevalent *spa* types demonstrated in MRSA cases, Denmark 2010

DANMAP 2010

spa type	CC group[a]	No. of cases	No. causing infections (%)
t002	CC5	110	67 (61)
t034	CC398	93	32 (34)
t008	CC8	90	63 (70)
t024	CC8	80	53 (66)
t019	CC30	73	59 (81)
t032	CC22	39	26 (67)
t044	CC80	34	27 (79)
t127	CC1	33	11 (33)
t041	CC22	25	10 (40)
t437	CC59	24	19 (79)

a) CC = Clonal complex

An EU funded research programme, PILGRIM, performed screening in 2010 for MRSA CC398 among pig farmers and their family, workers on pig farms and veterinarians. The number of cases from the targeted screening constituted 20 cases. t034 and other CC398 *spa* types were also seen in 15 cases without any known contact to pigs or other livestock, although the persons lived in areas with a high density of pig production facilities. They may represent an adaption of CC398 to the human host and the possibility of human-to-human spread. So far there are still no signs of spread through the food chain.

Resistance among MRSA isolates

The occurrence of resistance to tetracycline and mupirocin increased when comparing all MRSA isolates in 2010 with all MRSA isolates in 2009 (Table 8.10). The increase in tetracycline resistance reflects the increased number of t034 in 2010. The resistance pattern varied considerably between *spa* types (Table 8.10). In 2010, 100% of CC398 *spa* type t034 isolates were resistant to tetracycline and 100% of CC22 *spa* type t032 were resistant to norfloxacin.

In contrast, the majority of t019, a primarily community-acquired *spa* type, were susceptible to all tested antimicrobial agents except for beta-lactams. Even though differences in antimicrobial resistance were demonstrated between *spa* types, the success of antimicrobial treatment cannot be predicted based on *spa* type or epidemiological classification. Multi-resistance, defined as resistance to at least 1, 2 or 3 other antimicrobials in addition to cefoxitin/penicillin, was demonstrated in 72%, 58% and 38% of the cases, respectively. In Table 8.11, the most common resistance patterns and any frequent *spa* types are shown.

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Table 8.10. Resistance (%) in the six most prevalent *spa* types demonstrated in MRSA cases compared with all MRSA cases, Denmark 2010

DANMAP 2010

spa type	t002	t034	t008	t024	t019	t032	All cases
Clonal complex	CC5	CC398	CC8	CC8	CC30	CC22	
	%	%	%	%	%	%	%
Erythromycin	42	47	64	94	4	64	44
Clindamycin	39	77	7	88	3	64	38
Tetracycline	14	100	10	0	0	3	28
Fusidic acid	27	4	6	11	0	3	15
Rifampicin	4	0	0	0	1	0	3
Norfloxacin	34	19	55	24	1	100	31
Kanamycin	23	0	67	4	0	3	30
Linezolid	0	0	0	0	0	0	0
Mupirocin	1	0	1	0	0	3	3
Number of isolates	110	93	90	80	72	39	1097

Table 8.11. Resistance markers in addition to cefoxitin demonstrated in MRSA cases, Denmark 2010

DANMAP 2010

No. of markers	No. of cases	Frequent patterns (no. of isolates)	Any frequent *spa* type (no. of isolates)
0	306	–	–
1	150	T(41)	t034(17)
		N(38)	t032(12)
		F(35)	t002(12), t021(10)
		K(30)	
2	222	E,C(97)	t024(51), t002(16)
		C,T(25)	t034(19)
		F,N(22)	t002(14)
3	252	E,N,K(59)	t008(34), t657(14)
		E,C,N(52)	t032(24)
		E,C,T(49)	t034(35)
		T,F,K(40)	t044(20)
4	93	E,C,T,K(35)	t437(16), t127(8)
		E,C,N,K(22)	t002(8)
5	63	E,C,N,K,M(28)	t041(25)
		E,C,T,N,K(18)	t037(6), t189(5)
6	10	E,C,T,R,N,K(4)	
7	1	E,C,T,F,R,N,K(1)	t987(1)

a) T = tetracycline, N = norfloxacin, F = fusidic acid, K = kanamycin, E = erythromycin, C = clindamycin, R = rifampicin

Methicillin resistant *Staphylococcus aureus* (MRSA) in Danish pig herds, broilers and cattle at slaughter, and in Danish and imported retail meat

Background: Methicillin resistant *Staphylococcus aureus* (MRSA), especially belonging to the clonal complex CC398, has since 2003 emerged in livestock worldwide. The occurrence in Danish pigs at slaughter was investigated in 2009, and 13% of the pigs were found positive [DANMAP 2009]. As MRSA can be transmitted between animals during transportation and prior to slaughter, the occurrence found in the slaughterhouses may not be equivalent to the occurrence of MRSA positive farms [Broens *et al*. Vet J. 2010 (ahead of print)]. MRSA has also been found in many other animal species and in 2009, MRSA CC398 was found in a Danish beef sample. MRSA has not previously been found in cattle in Denmark. The aim of this study was to investigate the occurrence of MRSA at the pig farm level and to see if this differed significantly from that found at slaughter in 2009. We also wanted to investigate whether MRSA could be found in cattle and broilers at slaughter. Meat samples were collected to follow changes in occurrence when compared to data from 2009.

Materials and methods: During June through November 2010, pools of five nasal swab samples (n = 99) were taken from five slaughter pigs in five different pens in 99 farms in Denmark. Cattle, mainly young bulls, were tested at slaughter by taking skin swabs between leg and udder/testis of 192 animals, representing at least 174 different farms. One hundred ninety-seven pools of five throat swabs from broilers at the same farm were tested. Meat samples of Danish origin: pork (n = 183), broiler meat (n = 186), and beef (n = 118) as well as imported: pork (n = 176), broiler meat (n = 225), and beef (n = 99) were collected in retail stores and outlets. The meat samples were collected randomly in all regions of Denmark. MRSA was isolated from each pool of nasal/throat swabs, skin swabs or from 25 g of meat after pre-enrichment in Mueller-Hinton medium with 6.5% NaCl followed by selective enrichment in tryptone soya broth supplemented with 4 mg/L cefoxitin and 75 mg/L aztreonam. Ten µl were transferred to brilliance MRSA agar and colonies with typical *S. aureus* morphology were confirmed to be MRSA by PCR and the isolates were *spa* typed.

Results and discussion: Sixteen (16%) of the pig farms were positive for MRSA. All isolates except one were *spa* typed and all 15 isolates had *spa* types corresponding to CC398. The occurrence of MRSA in pig farms was not significantly different from what was found in pigs at slaughter in 2009 (13%). In 2009, 95% had *spa* types corresponding to CC398.

No MRSA were found among the 192 cattle and 197 broilers at slaughter. A study of MRSA in broilers using a similar sampling method found 6.9% MRSA positive broilers in The Netherlands [Mulders *et al*. Epidemiol Infect. 2010. 38: 743–55]. It can therefore be concluded that the occurrence of MRSA in Danish broilers is lower/absent compared to the Netherlands.

The absence of MRSA in cattle may be due to sampling method or that MRSA is not present. A Dutch study found that testing for MRSA by use of skin swabs between udder and legs of dairy cows was the most efficient method compared to testing of nasal swabs or milk. The use of skin swabs between udder/testis as in the present study may not have been so efficient, since the slaughter cattle were mainly young bulls and this sampling method has been tested for dairy cows (personal communication).

From meat samples, the highest occurrence of MRSA was found in imported broiler meat (19%), followed by Danish pork (6.0%), imported pork (5.7%) and imported beef (4.0%). No MRSA were found in Danish broiler meat or Danish beef. All except two isolates from meat were *spa* typed. From imported broiler meat, 89% corresponded to CC398, one isolate corresponded to CC5, one to CC7 and three corresponded to a new *spa* type. From Danish pork, only CC398 was found; from imported pork, CC398 was predominant, one isolate corresponded to CC1 and three isolates had a *spa* type not previously found. From imported beef, three isolates corresponded to CC398 and one isolate corresponded to CC7 (Figure 1).

MRSA CC398 was found in 109 human cases in 2010, the majority in persons with close contact to pigs or being a household member to a person with close contact to pigs. In 15 cases no such direct contact could be found - the majority of these were in persons living in rural areas with known occurrence of MRSA CC398 in pigs. There are still no sign of spread of CC398 to urban areas.

Conclusions: This investigation showed that 16% of Danish pig farms were MRSA positive, this is at approximately the same level as found in 2009 for pigs at slaughter. The occurrence of farms positive for MRSA is therefore still much lower compared to pig farms in The Netherlands and some other European countries. No MRSA was found in Danish broilers or cattle. In meat, MRSA was often found in imported broiler meat (19%), but not in Danish broiler meat. In pork, MRSA was found at the same level in Danish and imported meat (5-6%). In beef, MRSA was detected in imported but not in Danish meat. The relatively frequent occurrence of MRSA in meat combined with no/very few cases in urban areas makes it safe to conclude that there is very little if any risk for meat being a risk for contracting MRSA CC398. Pigs still seem to be the most important reservoir for MRSA CC398.

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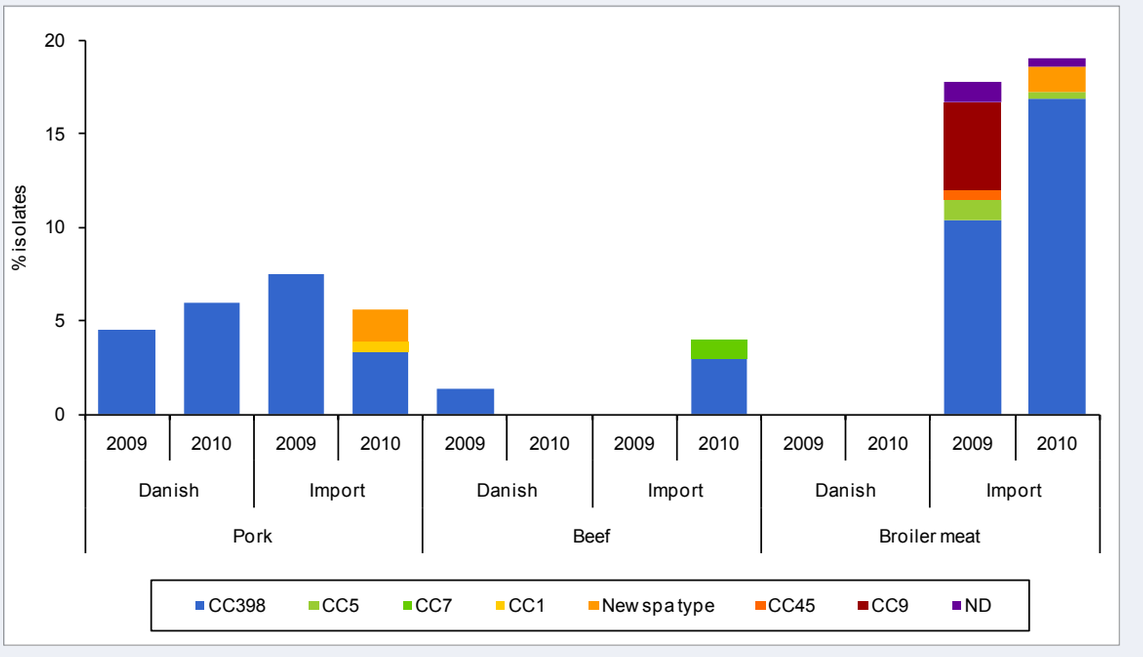
Figure 1. Occurrence (%) of MRSA in meat, Denmark

DANMAP 2010



Detection of a new *mecA* homologue in methicillin resistant *S. aureus* from human samples with a possible link to cattle

Methicillin resistance in *Staphylococcus aureus* (MRSA) is encoded by the *mecA* gene located on a mobile element called Staphylococcal Cassette Chromosome (SCC). Detection of the *mecA* gene is therefore "the golden standard" for MRSA confirmation. A new *mecA* homologue was however recently discovered by a group in Cambridge headed by Professor Mark Holmes. It was designated *mecAlga251*, according to the strain Lga251 in which it was found. The Lga251 isolate originated from bulk milk and a second milk isolate carrying mecAlga251 was also found. Another 13 isolates with *mecAlga251*were found in a collection of 940 bovine isolates and a subsequent survey in humans revealed the first identified human isolates in England.

Except for betalactams, the *mecAlga251* containing isolates are in general susceptible to other antimicrobials (gentamicin, neomycin, ciprofloxacin, tetracycline, erythromycin, clindamycin, fusidic acid, rifampicin and teicoplanin). The *mecAlga251* gene is located in a novel Staphylococcal Cassette Chromosome designated SCC*mec* XI and shares only 60% nucleotide homology with the conventional *mecA* gene. The low homology means that the isolates have been unrecognised by published MRSA PCRs and genotypic detection systems GeneOhm™ StaphSR (Becton Dickinson), GeneXpert™ MRSA (Cepheid) and NucliSENS EasyQ MRSA (bioMérieux) [Oliveira *et al.* 2002. Antimicrob Agents Chemother. 46: 2155–61; Kondo *et al.* 2007. Antimicrob Agents Chemother. 51: 264–74]. Likewise, the latex agglutination assays directed against PBP2a fail to detect the protein encoded by *mecAlga251*.

In Denmark, we became aware of the new gene in January 2011 after searching the isolate collections at SSI to identify isolates carrying the gene. The search included isolates being phenotypically resistant to cefoxitin but *mecA* negative from the years 2004 through 2010 (n = 149). Among these strains previously determined as borderline resistant (BORSA) or modified resistant (MODSA), we found 67 isolates to carry the *mecAlga251* gene. Furthermore, searching our bacteremia database (including data collected since 1958 on more than 40,000 isolates) revealed a curiosum: a *mecAlga251* positive strain dating back to 1975.

The geographic distribution of the isolates is shown in Table 1. Five persons were detected in the same Department of Clinical Microbiology (Slagelse) in 2010 and person to person transmission seems very likely. Isolates in which *mecAlga251* have been found belong to four genetic lineages within clonal complex 130: *spa* type t847 being the predominant. CC130 isolates have not been found in humans before but have previously been associated with bovine samples [Sung *et al.* 2008. Microbiology. 154: 1949–59]. We have not obtained any clinical information indicating that the affected Danish patients have had any contact to cattle.

Future studies should be addressed to elucidate the possible link between cattle and humans of this new *mecA* analogue, since so far no isolates from Danish cows have been detected.

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Table 1. *mecAlga251* cases per Department of Clinical Microbiology, Denmark DANMAP 2010

Department of Clinical Microbiology	2004	2005	2006	2007	2008	2009	2010	Total
Slagelse	3		2	1		1	5	12
Aalborg		1	2	3	1	1	2	10
Vejle		2	2			1	3	8
Sønderborg			2		1	2	1	6
Herlev		2	1					3
Næstved	2	1		1	1			5
Århus			2				3	5
Herning							3	3
Hillerød	1	1					2	4
Viborg				1	1	1		3
Esbjerg		2						2
Odense					1		1	2
SSI		1				1		2
Hvidovre			1					1
Nykøbing F.							1	1
Denmark total	6	10	12	6	5	7	21	67

9 RESISTANCE IN DIAGNOSTIC SUBMISSIONS FROM ANIMALS

Resistance in diagnostic submissions from animals

The DANMAP programme monitors antimicrobial resistance in *Escherichia coli* O149 from diagnostic submissions from pigs, and *E. coli* F5 (K99) from diagnostic submissions from cattle. *E. coli* was isolated from faecal samples, typically from pigs or calves with diarrhoea. The number of isolates available at the National Veterinary Institute has been decreasing annually due to outsourcing of the diagnostic tasks. In 2010, 33 *E. coli* (O149) were isolated from pigs and the distribution of MICs and occurrence of resistance in *E. coli* O149 from pigs is presented in Appendix 1 (Table AP1.24).

In 2010, only 14 cattle isolates of *E. coli* F5 (K99) were available and therefore these were not reported. *Staphylococcus hyicus* has not been reported since 2008 due to low number of available isolates.

9.1 *Escherichia* coli from pigs

Trends in resistance to selected antimicrobial agents in *E. coli* O149 isolates from pigs are presented in Figure 9.1. The isolates were mainly from weaning pigs with diarrhoea (>7.5 to 30 kg). Most isolates from diagnostic submissions originated from animals in antimicrobial therapy or with a history of recent antimicrobial therapy. For this reason, a higher frequency of resistance is expected in bacteria from diagnostic submissions compared to bacteria originating from healthy animals sampled at slaughter. In 2010, only one isolate (3%) was fully susceptible.

As in previous years, high levels of resistance (70%–80%) were found to tetracycline, sulfonamide and streptomycin. Sulfonamide and streptomycin are not used for weaning pig diarrhoea, and the consumption in weaning pigs has been stable at a low level (Figure 4.3); however, the resistance to these agents may be co-selected with tetracycline resistance, as tetracyclines are the most commonly used antimicrobials for weaning pigs (Figure 4.4).

In 2010, the only significant increase was seen for ciprofloxacin and nalidixic acid resistance, with 24% resistance to ciprofloxacin in 2010; all but two isolates (i.e. 6/8 isolates) were also resistant to the commonly used antimicrobials, tetracyclines and extended spectrum penicillins. The consumption of fluoroquinolones has been at a very low level in the pig production since 2003, but the resistance persists due to co-selection.

In 2010, one *E. coli* O149 isolate was resistant to cefotaxime and ceftiofur.

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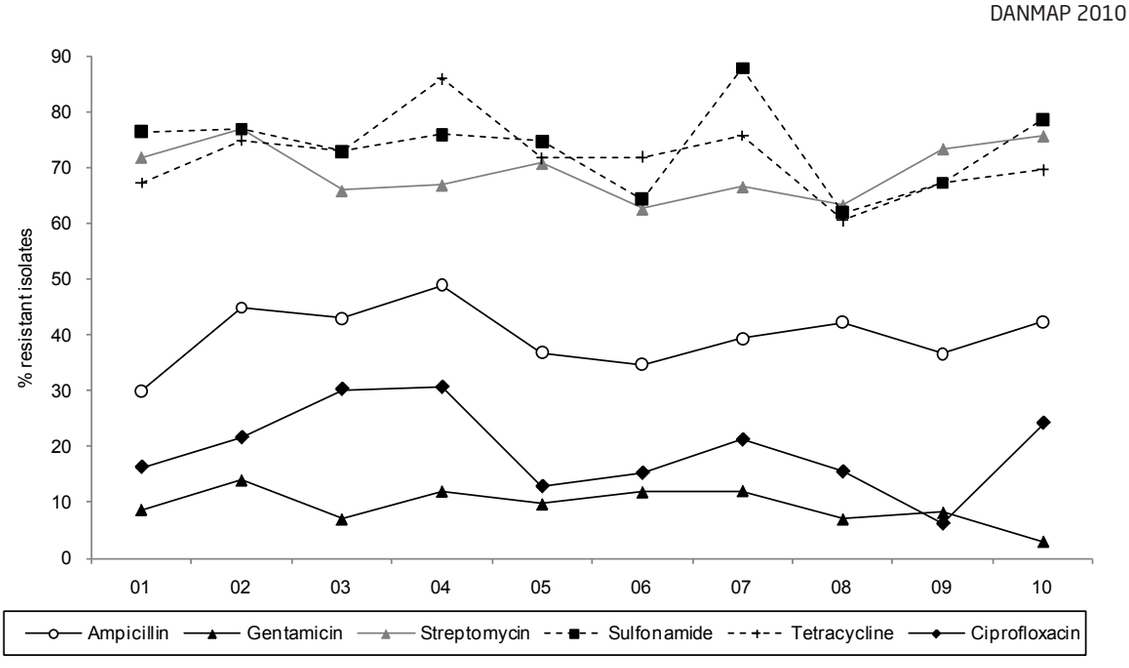
Figure 9.1. Resistance (%) in *Escherichia coli* O149 from diagnostic submissions from pigs, Denmark



1 APPENDIX


3%
11%
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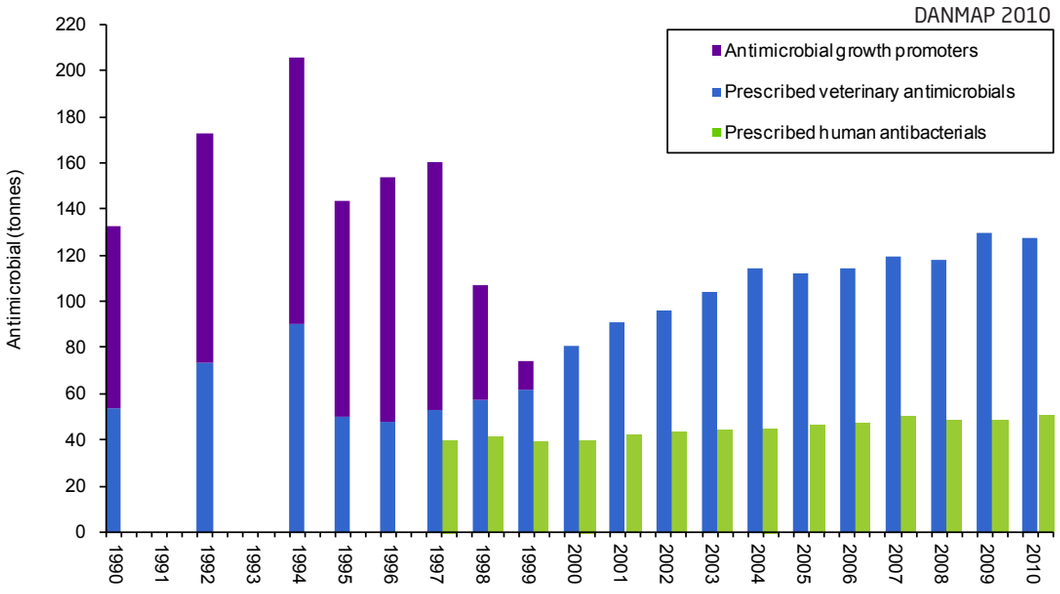
Figure AP1.1. Consumption of antimicrobial agents and growth promoters in animal production and prescribed antibacterials in humans, Denmark



Sources: Human therapeutics: The Danish Medicines Agency. Veterinary consumption: 1990–2000, data based on reports from the pharmaceutical industry of total annual sales. (Data 1990–1994: Use of antibiotics in the pig production. Federation of Danish pig producers and slaughterhouses. N. E. Rønn (Ed.). 1996–2000: Danish Medicines Agency and Danish Plant Directorate). 2001–2009: Data from VetStat.

Table AP1.0. Estimated total consumption (kg active compound) of prescribed antimicrobials for production animals 1990-2000, Denmark

DANMAP 2010

ATCvet group[a]	Therapeutic group	1990	1992	1994	1995	1996	1997	1998	1999	2000
QJ01AA	Tetracyclines	9300	22000	36500	9000	12900	13700	12100	16200	24000
QJ01CE	Penicillins, b-lactamase sensitive	5000	6700	9400	8800	7200	11200	14300	14700	15100
QJ01C/ QJ01D	Other penicillins, cephalosporins	1200	2500	4400	4500	5800	6100	6700	6600	7300
QJ01EW	Sulfonamides + trimethoprim	3800	7900	9500	6300	4800	6900	7700	6800	7000
QJ01EQ	Sulfonamides	8700	5900	5600	1800	2100	1400	1000	1000	1000
QJ01F	Macrolides, lincosamides, pleuromutilins	10900	12900	11400	9500	7600	6600	7100	8700	15600
QJ01G/ QA07AA	Aminoglycosides, colistin	7700	8500	8600	7600	7100	6100	7800	7500	10400
	Others[b]	6700	6800	4400	2100	600	650	650	350	300
Total		53300	73200	89800	49600	48100	52800	57350	61900	80700

Data based on reports from the pharmaceutical industry of total annual sales. 1990–1994: Data on use of antibiotics in the pig production. Federation of Danish pig producers and slaughterhouses. N. E. Rønn (Ed.). 1996–2000: Danish Medicines Agency. Only veterinary drugs are included. Veterinary drugs almost exclusively used in pets (tablets, capsules, ointment, eye/ear drops) are excluded

a) Only the major contributing ATCvet groups are mentioned. Kg active compound rounded to nearest 50 for antimicrobial classes and 100 for totals

b) Consumption in aquaculture was only partially included before 2001

Table AP1.1. Consumption of antimicrobial agents[a] for systemic use in pigs given as Animal Daily Doses (ADDs)[b], Denmark

DANMAP 2010

ATCvet code	QJ01AA	QJ01BA	QJ01CE	QJ01CA QJ01CR	QJ01DC QJ01DD	QJ01E	QJ01FA	QJ01FF	QA07AA	QA07AA10	QJ01MA	QJ01RA	QJ01XX	
Therapeutic group	Tetracyclines	Amphenicols	Penicillin's, b-lactamase sensitive	Amino-penicillins [c]	Cephalosporin[d]	Sulfonamides trimethoprim	Macrolides	Lincosamides / spectinomycin [e]	Aminoglycosides (local GI)	Colistin (local GI)	Fluoroquinolones	Penicillin streptomycin combinations	Pleuromutilin's	Total
Year	**Sows/piglets (1000's ADD200)**													
2001	1046	0	1574	719	38	803	763	428	291	1	93	597	533	6886
2002	1072	0	1793	894	60	965	764	555	252	26	51	643	498	7574
2003	1104	8	2039	993	99	1116	690	568	234	35	23	703	953	8567
2004	1135	9	2256	1080	113	1269	719	580	215	35	3	669	1027	9110
2005	1092	10	2344	1059	132	1366	724	567	167	35	4	661	845	9006
2006	1232	9	2371	1056	149	1434	780	542	152	35	7	647	955	9368
2007	1697	10	2589	1184	244	1568	1315	615	101	47	6	662	1300	11338
2008	1660	11	2647	1195	300	1635	1242	558	38	57	0	631	1842	11814
2009	1764	31	2865	1404	219	2033	1355	535	48	85	0	685	1726	12751
2010	1620	45	2796	1462	114	2092	1320	447	55	92	0	694	1287	12023
	Weaner pigs (1000's ADD15)													
2001	36163	0	2249	7158	60	3446	48410	13187	27324	75	531	1933	15230	155766
2002	31476	4	2552	8308	147	3987	44195	16575	23752	3172	188	2152	18255	154763
2003	32349	112	3015	10654	254	4185	39308	18691	22032	4377	17	2211	19779	156984
2004	39194	141	4144	13899	263	5516	49768	21189	21288	4531	8	3075	24984	188001
2005	45858	96	4258	12115	267	6192	48252	18269	19633	3994	5	3588	26747	189272
2006	56166	48	4050	10017	291	4698	46666	15881	19464	4212	11	3513	25496	190514
2007	76701	90	4472	9914	407	4192	54522	16203	10586	5299	0	3439	22655	208481
2008	83718	256	4144	9730	400	4559	51676	16597	2857	6727	0	3445	30834	214943
2009	98866	149	4618	11902	358	4668	59205	17823	2981	6862	0	3782	39241	250456
2010	91435	122	4775	11361	181	3939	56090	16636	2169	7349	0	4018	41232	239305
	Finisher pigs (1000's ADD50)													
2001	9223	0	4149	1505	16	173	10840	3131	262	1	129	296	7047	36773
2002	8936	0	4630	1756	36	206	11027	3693	220	22	69	351	7568	38515
2003	11492	30	5249	1995	56	177	11605	4233	192	28	6	423	8522	44008
2004	12689	43	6502	2835	60	237	11599	4447	124	22	4	380	10371	49313
2005	14074	35	7488	2674	62	247	12033	4223	236	20	2	368	12121	53582
2006	16231	33	7702	2275	50	159	10316	3524	213	27	1	297	10846	51673
2007	19320	20	7917	2155	54	172	10362	3194	109	20	0	226	8806	52354
2008	18824	20	7544	1547	53	152	10006	2637	5	43	0	158	12993	53983
2009	20000	16	8195	1651	39	120	11823	2737	13	30	0	129	15194	59948
2010	19581	10	8991	1671	22	112	11942	2695	38	32	0	210	16353	61657
	Age group not given (1000's ADD50)													
2001	1137	0	556	424	9	268	1471	545	584	0	89	139	806	6030
2002	800	2	444	296	7	202	929	330	209	22	20	82	630	3975
2003	768	5	491	305	9	210	951	376	149	39	0	98	676	4077
2004	915	7	557	289	9	154	1125	419	170	29	3	69	986	4731
2005	874	4	563	276	10	184	841	324	85	32	0	85	729	4007
2006	1168	2	510	315	11	177	755	279	144	34	0	69	722	4187
2007	675	1	254	101	11	84	369	186	48	27	0	26	395	2177
2008	398	1	147	94	9	56	235	90	8	35	0	8	287	1368
2009	233	0	110	78	10	43	205	56	2	24	0	10	187	958
2010	83	1	35	34	3	12	114	35	3	7	0	10	85	423

a) Data includes sales from pharmacies and feed mills. Consumption in veterinary practice comprises less than 1% of the total consumption in pigs and are not included, except for the use of fluoroquinolones. Local intrauterine and intramammary use is not included, and comprised less than 0.1‰ of the ADDs used in sows. Topical treatment is not included

b) Animal Standard weight is an assumed average weight at treatment, used to calculate numbe of ADD (Animal Daily Doses giving an estimated number of animals treated) from number of ADDkg (mass of animal treated, measured in kg animal bodyweight)

c) Includes a small proportion (< 1‰) of combinations with aminopenicillin and clavulanic acid

d) 3rd and 4th generation cephalosporins

e) Lincosamides and combnations between spectinomycin and lincosamides

Table AP1.2. Consumption of antimicrobial agents[a] for systemic use in cattle given as Animal Daily Doses (ADDs)[b], Denmark

DANMAP 2010

ATCvet code	QJ01AA	QJ01BA	QJ01CA QJ01CR	QJ01CE	QJ01DC QJ01DD	QJ01E	QJ01FA	QJ01FF	QA07AA	QA07AA10	QJ01MA	QJ01RA	
Therapeutic group	Tetracyclines	Amphenicols	Amino-penicillins [c]	Penicillin's, b-lactamase sensitive	Cephalosporin [d]	Sulfonamides and trimethoprim	Macrolides	Lincosamides / spectinomycin [e]	Aminoglycosides (local GI)	Colistin (local GI)	Fluoroquinolones	Penicillin streptomycin combinations	Total
Year	**Cows and bulls (1000's ADD600)**												
2005	186	1	58	490	71	65	112	2	19	0	0	22	1027
2006	193	1	57	498	64	61	116	2	9	0	0	22	1021
2007	235	1	68	610	79	73	91	2	2	0	0	28	1189
2008	257	1	80	702	85	75	65	1	1	0	0	34	1302
2009	279	2	84	804	73	73	53	1	2	0	0	36	1407
2010	269	1	79	835	70	73	38	0	2	0	0	42	1410
	Calves (1000's ADD100)												
2005	574	61	193	170	33	162	562	19	127	39	2	142	2083
2006	534	67	145	180	30	141	879	13	108	7	1	136	2242
2007	561	96	131	183	37	154	881	16	92	8	1	131	2290
2008	528	129	105	168	30	133	804	13	77	11	0	113	2111
2009	556	150	102	173	22	166	768	9	95	10	0	117	2167
2010	615	180	123	166	20	193	475	12	100	15	0	120	2018
	Heifers and steer (1000's ADD300)												
2005	18	0	5	27	3	3	8	1	0	0	0	2	67
2006	19	0	3	26	3	3	9	0	0	0	0	3	67
2007	24	1	6	33	4	3	10	2	0	0	0	4	86
2008	26	1	5	36	4	3	9	2	0	0	0	4	90
2009	26	1	5	37	3	3	6	1	0	0	0	5	88
2010	25	1	5	37	3	4	5	0	0	0	0	5	86
	age group unknown (1000's ADD600)												
2005	7	0	4	5	1	2	6	1	2	0	0	1	29
2006	21	1	13	14	2	4	31	6	5	1	0	2	99
2007	16	0	5	13	2	2	13	2	1	0	0	2	57
2008	2	0	1	3	1	0	2	0	0	0	0	0	10
2009	1	0	0	3	0	0	1	0	0	0	0	0	6
2010	1	0	0	4	0	1	1	0	0	0	0	0	7

a) Data includes sales from pharmacies and use for cattle in veterinary practice, including sales to the farmer. The consumption in calves is underestimated by up to 5% and consumption in cows is underestimated by up to 17% in individual years, because the use in cattle practice was underestimated by up to 20%. This error was decreasing with time (10% underestimation in 2010). Therefore, the numbers not fully representatrends over years, but reflects the choice of drug in individual years.

b) Animal Standard weight is an assumed average weight at treatment, used to calculate numbe of ADD (Animal Daily Doses giving an estimated number of animals treated) from number of ADDkg (mass of animal treated, measured in kg animal bodyweight)

c) Includes a small proportion (< 1‰) of combinations with aminopenicillin and clavulanic acid

d) 3rd and 4th generation cephalosporins

e) Lincomycin and lincomycin/spectinomycin combinations

Table AP1.3. Consumption of antimicrobial agents for systemic use in poultry given as Animal Daily Doses (ADDkg)[a], Denmark

DANMAP 2010

ATCvet code	QA07AA	QJ01A	QJ01CA	QJ01CE	QJ01E /QP51AG	QJ01FA	QJ01MA	QJ01X	QA07 /QJ01			
Therapeutic group	Aminoglycosides	Tetracyclines	Amoxicillin	Penicillins, b-lactamase sensitive	Sulfonamides[b]	Macrolides	Fluoroquinolones	Pleuromutilins	Others[c]	**Total**	Million kg meat or eggs[d]	ADD_{kg} per kg meat produced
Broilers (1000's ADDkg)												
2001	0	36	2777	0	90	16	250	13	0	**3181**	192	0.03
2002	0	0	3352	0	69	0	680	0	0	**4101**	190	0.04
2003	0	70	3052	0	8	0	270	0	0	**3399**	181	0.03
2004	100	116	4617	8	43	44	650	75	46	**5699**	181	0.07
2005	0	32	3984	22	58	3	661	0	100	**4860**	180	0.05
2006	0	0	3356	6	40	0	620	0	6	**4029**	163	0.06
2007	0	0	1718	0	168	289	130	0	36	**2341**	178	0.03
2008	0	429	4086	0	83	133	20	0	80	**4830**	186	0.07
2009	0	5200	6988	439	75	560	20	60	80	**13422**	181	0.15
2010	0	5469	13543	1158	135	522	0	0	20	**20846**	187	0.14
Rearing for broiler production (1000's ADDkg)												
2001	0	0	1392	0	30	0	230	0	0	**1652**	-	
2002	0	88	2025	0	96	0	660	0	0	**2869**	-	
2003	0	0	1361	0	0	0	80	0	0	**1441**	-	
2004	0	0	6464	0	0	0	490	0	0	**6954**	-	Included in broiler production above
2005	0	0	3348	0	0	0	400	0	0	**3748**	-	
2006	0	0	6238	0	15	0	114	0	0	**6367**	-	
2007	0	0	2659	0	43	22	190	0	0	**2914**	-	
2008	0	400	6913	0	100	322	0	0	10	**7745**	-	
2009	0	2067	7738	2851	80	289	440	0	290	**13754**	-	
2010	0	2267	2825	719	44	33	0	0	0	**5888**		
Layers and layer rearing (1000's ADDkg)[d]												
2001	0	19	434	0	196	16	50	13	0	**727**	69	0.01
2002	0	285	670	0	171	0	100	0	0	**1226**	70	0.02
2003	0	540	350	0	328	0	0	0	0	**1218**	69	0.02
2004	0	2	819	2	215	6	30	0	230	**1303**	72	0.02
2005	0	8	680	4	243	0	0	3	30	**967**	69	0.01
2006	0	28	376	0	140	11	0	0	0	**555**	67	0.01
2007	0	0	1150	0	96	0	0	0	150	**1396**	67	0.02
2008	0	12	2563	0	100	0	0	0	70	**2745**	68	0.04
2009	0	713	1475	0	15	2	0	0	488	**2693**	61	0.04
2010	0	133	1488	0	8	171	0	275	395	**2469**	62	0.04
Turkeys (1000's ADDkg)												
2001	0	0	10477	0	0	0	90	0	0	**10567**	13.2	0.80
2002	0	0	26829	0	0	0	0	0	0	**26829**	12.8	2.10
2003	0	0	10900	0	58	0	360	4568	0	**15885**	11.2	1.42
2004	200	0	4873	0	76	16	1560	0	0	**6725**	19.6	0.34
2005	150	60	8963	0	68	0	780	0	0	**10020**	17.4	0.58
2006	100	150	15193	0	45	0	1160	0	0	**16648**	11.3	1.47
2007	518	1654	6788	278	0	2547	2430	0	728	**14941**	14.4	1.04
2008	0	5767	1038	0	4	811	190	0	531	**8340**	12.3	0.68
2009	0	11771	4563	491	0	2538	0	0	536	**19899**	11.1	1.79
2010	0	6119	300	0	86	1922	0	0	253	**8680**	14.0	0.62

Table AP1.3 (Continued). Consumption of antimicrobial agents for systemic use in poultry given as Animal Daily Doses (ADD_{kg})[a], Denmark

DANMAP 2010

ATCvet code	QA07AA	QJ01A	QJ01CA	QJ01CE	QJ01E /QP51AG	QJ01FA	QJ01MA	QJ01X	QA07 /QJ01			
Therapeutic group	Aminoglycosides	Tetracyclines	Amoxicillin	Penicillins, b-lactamase sensitive	Sulfonamides[b]	Macrolides	Fluoroquinolones	Pleuromutilins	Others[c]	**Total**	Million kg meat or eggs[d]	ADD_{kg} per kg meat produced
Ducks and geese (1000's ADDkg)												
2001	0	2	0	0	1	11	50	3	0	**67**	4.5	0.01
2002	0	12	36	0	0	30	0	0	0	**77**	4.9	0.02
2003	0	8	257	0	0	0	0	0	0	**265**	4.2	0.06
2004	0	14	400	0	13	11	150	3	0	**591**	4.2	0.14
2005	0	0	525	0	0	14	0	3	0	**542**	4.1	0.13
2006	0	0	1125	0	0	0	0	0	0	**1125**	4.5	0.25
2007	0	0	100	0	0	0	0	0	2	**102**	2.4	0.04
2008	0	36	250	0	1	0	0	0	0	**287**	2.6	0.11
2009	0	24	0	0	10	200	0	0	0	**234**	2.2	0.11
2010	0	914	0	0	3	0	0	0	0	**917**	2.0	0.45
Game birds (1000's ADDkg)												
2001	0	77	768	0	205	146	85	5	84	**1370**	-	-
2002	125	177	1466	0	346	289	10	10	94	**2518**	-	-
2003	150	128	923	0	318	273	1	933	0	**2725**	-	-
2004	250	148	1003	0	460	113	30	18	0	**2022**	-	-
2005	160	98	1939	0	403	177	0	13	14	**2803**	-	-
2006	110	86	1863	0	258	39	11	5	42	**2413**	-	-
2007	2	126	1425	0	542	37	0	0	73	**2203**	-	-
2008	110	80	1825	0	256	39	11	0	38	**2360**	-	-
2009	0	270	901	18	664	46	10	0	172	**2080**	-	-
2010	3	267	1083	0	1443	44	10	25	161	**3036**	-	-
Production type unknown[e] (1000's ADDkg)												
2001	1	155	3814	0	441	306	475	10	18	**5219**	-	-
2002	29	95	2909	0	315	272	93	5	0	**3718**	-	-
2003	300	91	2370	0	348	186	391	5	0	**3690**	-	-
2004	450	106	3654	0	440	90	131	3	4	**4878**	-	-
2005	0	58	2978	0	192	3	121	5	46	**3403**	-	-
2006	50	144	3059	0	182	4	110	0	0	**3549**	-	-
2007	0	140	1321	72	518	118	34	8	58	**2267**	-	-
2008	0	374	863	0	263	148	3	3	39	**1692**	-	-
2009	2	794	486	0	182	22	11	5	56	**1557**	-	-
2010	0	142	97	0	85	11	12	3	14	**363**	-	-

a) ADDkg is the dose necessary for treating 1 kg body-weight

b) Includes sulfaclozin (a coccidiostat/antibacterial) and sulfonamide/trimethoprim combinations

c) Includes QA07AA10 (colistin), QJ01FF (lincosamides, including combinations with spectinomycin), QJ01B (amphenicols) and QJ01R (penicillin/streptomycin combinations)

d) For layers and layer rearing, only the production of eggs for consumption is included (not the slaughter/export of hens)

e) Includes prescription with erraneous farm id or farms with more than one poultry species; for 2009–2010 this was mainly pigeons and game birds.

Table AP1.4. Consumption of antibacterial agents for systemic use in primary health care (No. packages/1000 inhabitants/year), Denmark

DANMAP 2010

ATC group[a]	Therapeutic group	Year									
		2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
J01AA	Tetracyclines	22.4	21.7	21.6	22.5	23.8	23.9	24.5	25.0	25.9	27.2
J01CA	Penicillins with extended spectrum	110.9	111.8	111.5	115.3	119.9	119.7	131.3	130.0	130.2	140.2
J01CE	Beta-lactamase sensitive penicillins	251.0	254.4	254.5	253.7	251.1	243.3	253.0	235.9	223.2	228.2
J01CF	Beta-lactamase resistant penicillins	30.1	37.5	41.9	43.0	44.4	44.0	45.8	45.4	45.2	45.9
J01CR	Combinations of penicillins, including beta-lactamase inhibitors	1.2	1.7	2.0	2.5	3.0	4.0	5.8	8.0	12.3	18.0
J01D	Cephalosporins and related substances	1.3	1.4	1.3	1.4	1.6	1.7	1.8	2.1	2.1	2.1
J01EA	Trimethoprim and derivatives	8.2	8.8	9.3	10.2	10.6	10.7	11.5	12.4	10.9	11.3
J01EB	Short-acting sulfonamides	47.8	47.6	47.9	48.3	47.5	45.8	41.0	36.0	34.6	34.3
J01EE	Combinations of sulfonamides and trimethoprim, including derivatives	1.4	1.3	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
J01FA	Macrolides	102.2	102.8	99.8	102.7	110.3	101.8	108.6	103.3	99.6	110.5
J01FF	Lincosamides	0.5	0.6	0.6	0.7	1.1	1.4	1.6	2.0	2.5	2.8
J01GB	Aminoglycosides	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
J01MA	Fluoroquinolones	10.6	11.0	13.8	16.2	18.3	19.4	22.9	25.1	25.0	27.4
J01XA	Glycopeptides	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.2	0.2	0.4
J01XB	Polymyxins	2.1	2.0	2.0	2.1	2.0	1.5	0.8	0.8	0.9	0.9
J01XC	Steroid antibacterials (fusidic acid)	0.8	0.8	0.7	0.6	0.7	0.7	0.7	0.8	0.7	0.7
J01XE	Nitrofuran derivatives (nitrofurantoin)	10.4	11.1	11.3	11.7	12.3	12.5	11.9	12.2	12.6	12.4
J01XX05	Methenamine	3.2	3.2	2.6	2.4	2.3	2.0	1.9	2.0	1.9	1.9
J01XX08	Linezolid	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
J01	Antibacterial agents for systemic use (total)	604.4	618.0	622.3	633.6	649.3	632.6	663.5	641.2	628.0	664.4

a) From the 2010 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Table AP1.5. Consumption of antibacterial agents for systemic use in primary health care (No. treated patients/1000 inhabitants/year), Denmark

DANMAP 2010

ATC group[a]	Therapeutic group	Year									
		2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
J01AA	Tetracyclines	11.8	11.5	11.4	11.6	12.0	12.3	12.5	12.7	13.0	13.4
J01CA	Penicillins with extended spectrum	69.4	69.2	68.8	70.6	73.0	75.8	82.1	81.3	81.1	85.1
J01CE	Beta-lactamase sensitive penicillins	173.3	173.4	172.6	171.2	170.2	171.3	177.1	164.4	158.8	162.9
J01CF	Beta-lactamase resistant penicillins	19.2	23.9	26.4	27.1	27.8	29.4	29.7	29.9	29.9	30.0
J01CR	Combinations of penicillins, including beta-lactamase inhibitors	0.7	1.0	1.1	1.3	1.5	2.3	3.6	5.0	8.0	11.7
J01D	Cephalosporins and related substances	0.4	0.4	0.4	0.5	0.5	0.5	0.5	0.5	0.5	0.5
J01EA	Trimethoprim and derivatives	4.2	4.5	4.6	5.0	5.4	5.6	5.9	5.9	5.8	6.0
J01EB	Short-acting sulfonamides	33.2	33.0	33.1	33.3	32.7	33.0	29.7	26.3	25.4	25.0
J01EE	Combinations of sulfonamides and trimethoprim, including derivatives	0.8	0.7	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0
J01FA	Macrolides	67.7	66.9	64.1	65.9	70.7	67.0	71.4	66.9	64.5	72.7
J01FF	Lincosamides	0.2	0.3	0.3	0.4	0.4	0.5	0.6	0.8	1.0	1.3
J01GB	Aminoglycosides	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
J01MA	Fluoroquinolones	7.5	7.7	8.9	10.8	12.2	13.1	15.2	17.1	16.9	18.5
J01XA	Glycopeptides	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
J01XB	Polymyxins	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
J01XC	Steroid antibacterials (fusidic acid)	0.5	0.4	0.3	0.3	0.3	0.4	0.3	0.3	0.3	0.3
J01XE	Nitrofuran derivatives (nitrofurantoin)	5.7	6.1	6.2	6.4	6.7	7.0	6.5	6.8	7.0	6.9
J01XX05	Methenamine	0.5	0.6	0.5	0.5	0.5	0.4	0.4	0.4	0.4	0.4
J01XX08	Linezolid	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
J01[b]	Antibacterial agents for systemic use (total)	300.6	301.5	301.4	302.6	308.0	310.3	320.4	308.2	303.1	315.5

a) From the 2010 edition of the Anatomical Therapeutic Chemical (ATC) classification system
b) Total no. of patients treated with an antibiotic is lower than the sum of all antibiotic classes. This is because the Danish Medicines Agency only counts the first treatment for each patient, each year

Table AP1.6. Number of DDDs and packages per treated patient in primary health care, Denmark

DANMAP 2010

ATC group[a]	Therapeutic group	Indicator	Year									
			2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
J01AA	Tetracyclines	DDDs / patient	30.6	33.0	34.4	36.9	39.0	40.9	43.0	44.4	45.2	45.9
		DDDs / package	16.1	17.5	18.1	19.0	19.6	21.0	22.0	22.7	22.7	22.7
		Packages / patient	1.9	1.9	1.9	1.9	2.0	1.9	2.0	2.0	2.0	2.0
J01CA	Penicillins with extended spectrum	DDDs / patient	13.0	13.2	13.4	13.6	13.9	14.2	14.4	14.7	14.8	14.9
		DDDs / package	8.1	8.2	8.2	8.4	8.5	8.9	9.0	9.2	9.2	9.0
		Packages / patient	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6
J01CE	Beta-lactamase sensitive penicillins	DDDs / patient	10.3	10.5	10.7	11.1	11.3	11.5	11.7	11.8	11.8	11.8
		DDDs / package	7.1	7.2	7.3	7.5	7.7	8.0	8.2	8.2	8.4	8.4
		Packages / patient	1.4	1.5	1.5	1.5	1.5	1.4	1.4	1.4	1.4	1.4
J01CF	Beta-lactamase resistant penicillins	DDDs / patient	12.4	11.8	11.8	12.4	12.7	13.0	13.4	13.7	13.9	14.2
		DDDs / package	7.9	7.5	7.4	7.8	8.0	8.6	8.7	9.0	9.1	9.3
		Packages / patient	1.6	1.6	1.6	1.6	1.6	1.5	1.5	1.5	1.5	1.5
J01CR	Combinations of penicillins, incl. beta-lactamase inhibitors	DDDs / patient	15.9	14.7	16.6	17.2	16.8	19.3	19.1	19.9	20.4	21.1
		DDDs / package	9.1	8.6	9.1	9.1	9.3	10.7	11.7	12.4	13.3	13.7
		Packages / patient	1.7	1.7	1.8	2.0	2.0	1.8	1.6	1.6	1.5	1.5
J01D	Cephalosporins and related substances	DDDs / patient	25.5	24.9	18.3	18.6	21.7	20.7	21.9	23.8	22.7	24.7
		DDDs / package	8.4	7.8	5.6	6.1	6.2	5.8	6.1	5.8	5.7	5.8
		Packages / patient	3.0	3.2	3.3	3.0	3.5	3.5	3.6	4.1	4.0	4.3
J01EA	Trimethoprim and derivatives	DDDs / patient	30.4	29.3	30.0	29.9	30.2	30.6	30.5	30.2	30.7	30.7
		DDDs / package	15.6	14.9	14.9	14.8	15.3	15.9	15.7	14.5	16.1	16.4
		Packages / patient	2.0	2.0	2.0	2.0	2.0	1.9	1.9	2.1	1.9	1.9
J01EB	Short-acting sulfonamides	DDDs / patient	4.0	4.0	4.0	3.9	3.9	3.9	3.9	3.8	3.8	3.8
		DDDs / package	2.7	2.7	2.7	2.7	2.7	2.8	2.8	2.8	2.8	2.8
		Packages / patient	1.4	1.4	1.4	1.5	1.5	1.4	1.4	1.4	1.4	1.4
J01EE	Combinations of sulfonamides and trimethoprim. incl. derivatives	DDDs / patient	19.4	15.6	18.3	-	-	-	-	-	-	-
		DDDs / package	10.4	8.4	11	-	-	-	-	-	-	-
		Packages / patient	1.9	1.9	1.67	-	-	-	-	-	-	-
J01FA	Macrolides	DDDs / patient	11.3	11.7	12.1	12.4	12.4	12.6	12.4	12.5	12.5	12.2
		DDDs / package	7.5	7.6	7.8	7.9	8.0	8.3	8.1	8.1	8.1	8.1
		Packages / patient	1.5	1.5	1.6	1.6	1.6	1.5	1.5	1.5	1.5	1.5
J01FF	Lincosamides	DDDs / patient	15.2	11.1	11.1	13.9	13.4	13.8	13.3	12.8	12.6	11.4
		DDDs / package	7.3	6.1	6.1	7.6	4.9	4.8	4.9	5.0	5.0	5.2
		Packages / patient	2.1	1.8	1.8	1.8	2.8	2.9	2.7	2.5	2.5	2.2
J01GB	Aminoglycosides	DDDs / patient		121.7	121.7	156.5	172.2	135.6	128.0	152.7	157.6	151.5
		DDDs / package		18.3	36.5	47.0	51.7	27.1	26.0	32.2	37.8	43.4
		Packages / patient		6.7	3.3	3.3	3.3	5.0	4.9	4.9	4.2	3.5
J01MA	Fluoroquinolones	DDDs / patient	8.3	8.6	10.3	9.5	9.6	10.3	10.6	11.0	11.2	11.2
		DDDs / package	5.9	6.0	6.6	6.4	6.5	6.9	7.0	7.5	7.6	7.6
		Packages / patient	1.4	1.4	1.6	1.5	1.5	1.5	1.5	1.5	1.5	1.5
J01XB	Polymyxins	DDDs / patient	183	243.3	243.3	192.3	196.7	205.6	219.3	202.8	202.8	199.4
		DDDs / package	3.5	3.7	3.7	3.7	3.9	5.5	10.0	10.0	10.0	10.0
		Packages / patient	52.5	66.7	66.7	52.5	50.0	37.5	21.9	20.3	20.3	19.9
J01XC	Steroid antibacterials (fusidic acid)	DDDs / patient	7.6	8.7	11.1	14.4	16.0	15.1	17.1	18.5	18.7	18.8
		DDDs / package	4.6	4.6	5.2	7.2	7.6	7.6	8.0	7.3	6.8	7.7
		Packages / patient	1.7	1.9	2.1	2.0	2.1	2.0	2.1	2.5	2.8	2.4
J01XE	Nitrofuran derivatives (nitrofurantoin)	DDDs / patient	24.8	24.5	24.8	24.3	24.3	24.1	26.3	25.4	25.4	26.8
		DDDs / package	13.7	13.5	13.6	13.3	13.3	13.5	14.4	14.2	14.1	15.0
		Packages / patient	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8
J01XX05	Methenamine	DDDs / patient	227.3	225.6	220.4	221.6	222.9	233.1	237.5	239.9	227.2	234.1
		DDDs / package	37.6	38.8	44.9	45.2	44.6	49.0	50.1	50.0	50.0	50.0
		Packages / patient	6.0	5.8	4.9	4.9	5	4.8	4.7	4.8	4.5	4.7
J01	Antibacterial agents for systemic use (total)	DDDs / patient	15.6	16.0	16.4	17.0	17.5	17.9	17.3	18.9	19.2	19.6
		DDDs / package	7.8	7.8	7.9	8.1	8.3	8.7	8.9	9.1	9.3	9.3
		Packages / patient	2.0	2.0	2.1	2.1	2.1	2.0	1.9	2.1	2.1	2.1

a) From the 2010 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Table AP1.7. Activity at somatic hospitals, Denmark DANMAP 2010

Region	No. bed-days somatic hospitals[a]	No. admissions somatic hospitals[a]
The Capital Region of Denmark	1514665	450077
The Sealand Region	591364	210413
Region of Southern Denmark	839553	258316
Central Denmark Region	869636	278195
North Denmark Region	454664	117661
Denmark[b]	4269882	1314662

Source: The National Board of Health (www.sst.dk)

a) Excluding private hospitals, psychiatric hospitals, specialized clinics, rehabilitation centres and hospices

b) Compared to the previous year no. bed-days have decreased by 3.4% and no. admissions have increased by 4.0%

Table AP1.8. Consumption of antibacterial agents for systemic use in hospital care (DDD/1000 inhabitant-days), Denmark DANMAP 2010

ATC group[a]	Therapeutic group	Year									
		2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
J01AA	Tetracyclines	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.03	0.03
J01CA	Penicillins with extended spectrum	0.34	0.33	0.33	0.32	0.35	0.35	0.35	0.35	0.35	0.32
J01CE	Beta-lactamase sensitive penicillins	0.32	0.33	0.34	0.33	0.33	0.29	0.28	0.25	0.23	0.21
J01CF	Beta-lactamase resistant penicillins	0.18	0.18	0.18	0.19	0.18	0.18	0.18	0.17	0.17	0.17
J01CR	Combinations of penicillins, incl. beta-lactamase inhibitors	0.01	0.01	0.01	0.02	0.03	0.05	0.08	0.10	0.13	0.15
J01DB	First-generation cephalosporins	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
J01DC	Second-generation cephalosporins	0.15	0.17	0.17	0.19	0.22	0.23	0.31	0.33	0.37	0.35
J01DD	Third-generation cephalosporins	0.02	0.02	0.02	0.02	0.02	0.02	0.03	0.03	0.03	0.03
J01DF	Monobactams	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
J01DH	Carbapenems	0.01	0.02	0.02	0.02	0.03	0.03	0.05	0.07	0.07	0.08
J01EA	Trimethoprim and derivatives	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
J01EB	Short-acting sulfonamides	0.04	0.04	0.03	0.03	0.03	0.02	0.01	0.01	0.01	0.01
J01EE	Combinations of sulfonamides and trimethoprim, incl. derivatives	0.04	0.04	0.04	0.05	0.05	0.05	0.04	0.05	0.05	0.06
J01FA	Macrolides	0.10	0.09	0.09	0.08	0.08	0.08	0.08	0.08	0.08	0.08
J01FF	Lincosamides	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
J01GB	Aminoglycosides	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.04	0.04	0.04
J01MA	Fluoroquinolones	0.08	0.10	0.11	0.13	0.16	0.18	0.21	0.24	0.24	0.22
J01XA	Glycopeptides	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.02
J01XB	Polymyxins	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
J01XC	Steroid antibacterials (fusidic acid)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
J01XD	Imidazol derivatives	0.06	0.06	0.06	0.07	0.07	0.07	0.07	0.06	0.05	0.08
J01XE	Nitrofuran derivatives (nitrofurantoin)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
J01XX	Other antibacterials	0.00	0.01	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.01
J01	Antibacterial agents for systemic use (total)	1.45	1.51	1.51	1.56	1.67	1.70	1.81	1.87	1.91	1.91

a) From the 2010 edition of the Anatomical Therapeutic Chemical (ATC) classification system

Table AP1.9. Distribution of MICs and resistance (%) in *Salmonella* Typhimurium from cattle (n=18) and pigs (n=455), Denmark DANMAP 2010

Antimicrobial agent	Animal species	% Resistant	95% Confidence interval	Distribution (%) of MICs																		
				0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	>2048
Tetracycline	Cattle	61.1	[35.7-82.7]								33.3	5.6			5.6	55.6						
	Pigs	47.5	[42.8-52.2]								49.7	2.9			4.4	43.1						
Chloramphenicol	Cattle	5.6	[0.1-27.3]									61.1	33.3				5.6					
	Pigs	8.8	[6.4-11.8]								0.2	62.4	27.0	1.5	0.4	1.5	6.8					
Florfenicol	Cattle	5.6	[0.1-27.3]									77.8	16.7		5.6							
	Pigs	5.9	[3.9-8.5]								0.7	80.9	10.3	2.2	4.2	0.4	1.3					
Ampicillin	Cattle	55.6	[30.8-78.5]							27.8	11.1	5.6				55.6						
	Pigs	49.2	[44.5-53.9]							39.3	10.3	1.1				49.2						
Ceftiofur	Cattle	0	[0-18.5]						55.6	44.4												
	Pigs	0	[0-0.8]						49.5	44.2	6.4											
Cefotaxime	Cattle	0	[0-18.5]				94.4	5.6														
	Pigs	0	[0-0.8]				93.4	6.2	0.4													
Trimethoprim	Cattle	0	[0-18.5]							100												
	Pigs	8.4	[6.0-11.3]							91.6						8.4						
Sulfonamide	Cattle	55.6	[30.8-78.5]													44.4					55.6	
	Pigs	53.2	[48.5-57.8]													46.8					53.2	
Streptomycin	Cattle	66.7	[41.0-86.7]										5.6	27.8	11.1			55.6				
	Pigs	56.5	[51.8-61.1]										9.5	34.1	4.0	0.7	5.5	46.4				
Gentamicin	Cattle	0	[0-18.5]						38.9	61.1												
	Pigs	1.8	[0.8-3.4]						52.5	43.5	2.2	0.2		0.4	1.1							
Neomycin	Cattle	0	[0-18.5]								88.9	11.1										
	Pigs	3.1	[1.7-5.1]								92.7	4.2	0.2	0.2		2.6						
Apramycin	Cattle	0	[0-18.5]									77.8	22.2									
	Pigs	1.3	[0.5-2.8]									79.8	18.5	0.4		1.3						
Ciprofloxacin	Cattle	0	[0-18.5]		88.9	11.1																
	Pigs	0.2	[0.01-1.2]	4.2	87.7	7.9	0.2															
Nalidixic acid	Cattle	0	[0-18.5]									88.9	11.1									
	Pigs	0	[0-0.8]									83.5	14.9	1.5								
Colistin	Cattle	0	[0-18.5]							100												
	Pigs	0	[0-0.8]							99.6	0.4											
Spectinomycin	Cattle	5.6	[0.1-27.3]												55.6	38.9			5.6			
	Pigs	15.8	[12.6-19.5]											0.4	56.7	27.0	0.4	2.0	13.4			

Vertical solid lines indicate EUCAST epidemiological cut-off values except for apramycin, spectinomycin and sulfonamide where the cut-off values were set by DANMAP. EUCAST/CLSI clinical breakpoints indicated as vertical dotted lines if different from the corresponding epidemiological cut-off values. See table AP2.2 for further details

White fields represent the range of dilutions tested. MIC values equal to or lower than the lowest concentration tested are presented as the lowest concentration. MIC values greater than the highest concentration in the range are presented as one dilution step above the range

Table AP1.10. Distribution of MICs and resistance (%) in *Salmonella* Typhimurium from imported broiler meat (n=18), imported turkey meat (n=41) and pork (Danish n=26; imported n=62), Denmark

DANMAP 2010

Antimicrobial agent	Food type	Origin	% Resistant	95% Confidence interval	Distribution (%) of MICs																	
					0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	>2048
Tetracycline	Broiler meat	Imported	5.6	[0.1-27.3]								94.4					5.6						
	Turkey meat	Imported	100	[91.4-100.0]												12.2	87.8						
	Pork	Danish	26.9	[11.6-47.8]								73.1				3.8	23.1						
		Imported	77.4	[65.0-87.1]								22.6				6.5	71.0						
Chloramphenicol	Broiler meat	Imported	0	[0-18.5]									94.4	5.6									
	Turkey meat	Imported	9.8	[2.7-23.1]									63.4	24.4	2.4			9.8					
	Pork	Danish	3.8	[0.1-19.6]									69.2	26.9				3.8					
		Imported	21.0	[11.7-33.2]									46.8	32.3		1.6	9.7	9.7					
Florfenicol	Broiler meat	Imported	0	[0-18.5]									100										
	Turkey meat	Imported	9.8	[2.7-23.1]									63.4	26.8		9.8							
	Pork	Danish	3.8	[0.1-19.6]									92.3	3.8		3.8							
		Imported	9.7	[3.6-19.9]									74.2	16.1		8.1		1.6					
Ampicillin	Broiler meat	Imported	11.1	[1.4-34.7]							83.3	5.6					11.1						
	Turkey meat	Imported	68.3	[51.9-81.9]							7.3	24.4					68.3						
	Pork	Danish	34.6	[17.2-55.7]							46.2	19.2					34.6						
		Imported	72.6	[59.8-83.1]							22.6	4.8					72.6						
Ceftiofur	Broiler meat	Imported	0	[0-18.5]						61.1	38.9												
	Turkey meat	Imported	2.4	[0.06-12.9]						46.3	29.3	22.0			2.4								
	Pork	Danish	0	[0-13.2]						42.3	57.7												
		Imported	0	[0-5.8]						43.5	48.4	8.1											
Cefotaxime	Broiler meat	Imported	0	[0-18.5]				94.4	5.6														
	Turkey meat	Imported	2.4	[0.06-12.9]				87.8	9.8					2.4									
	Pork	Danish	0	[0-13.2]				92.3	7.7														
		Imported	0	[0-5.8]				91.9	6.5	1.6													
Trimethoprim	Broiler meat	Imported	0	[0-18.5]							100												
	Turkey meat	Imported	26.8	[14.2-42.9]							73.2						26.8						
	Pork	Danish	0	[0-13.2]							100												
		Imported	17.7	[9.2-29.5]							82.3						17.7						
Sulfonamide	Broiler meat	Imported	11.1	[1.4-34.7]													88.9					11.1	
	Turkey meat	Imported	92.7	[80.1-98.5]													7.3					92.7	
	Pork	Danish	38.5	[20.2-59.4]													61.5					38.5	
		Imported	83.9	[72.3-92.0]													16.1					83.9	
Streptomycin	Broiler meat	Imported	27.8	[9.7-53.5]										11.1	61.1	16.7			11.1				
	Turkey meat	Imported	92.7	[80.1-98.5]											7.3	2.4	14.6	12.2	63.4				
	Pork	Danish	46.2	[26.6-66.6]										19.2	34.6	7.7		3.8	34.6				
		Imported	87.1	[76.1-94.3]											12.9	14.5	1.6	8.1	62.9				

Table AP1.10 (Continued). Distribution of MICs and resistance (%) in *Salmonella* Typhimurium from imported broiler meat (n=18), imported turkey meat (n=41) and pork (Danish n=26; imported n=62), Denmark

DANMAP 2010

Antimicrobial agent	Food type	Origin	% Resistant	95% Confidence interval	Distribution (%) of MICs																	
					0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	>2048
Gentamicin	Broiler meat	Imported	0	[0-18.5]						55.6	33.3	11.1										
	Turkey meat	Imported	26.8	[14.2-42.9]						17.1	53.7	2.4				26.8						
	Pork	Danish	0	[0-13.2]						57.7	38.5	3.8										
		Imported	0	[0-5.8]						66.1	33.9											
Neomycin	Broiler meat	Imported	0	[0-18.5]								94.4	5.6									
	Turkey meat	Imported	24.4	[12.4-40.3]								63.4	12.2	24.4								
	Pork	Danish	0	[0-13.2]								96.2	3.8									
		Imported	3.2	[0.4-11.2]								91.9	4.8				3.2					
Apramycin	Broiler meat	Imported	0	[0-18.5]									77.8	22.2								
	Turkey meat	Imported	24.4	[12.4-40.3]									56.1	19.5			24.4					
	Pork	Danish	0	[0-13.2]									73.1	26.9								
		Imported	0	[0-5.8]									83.9	16.1								
Ciprofloxacin	Broiler meat	Imported	0	[0-18.5]	5.6	94.4																
	Turkey meat	Imported	2.4	[0.06-12.9]		68.3	29.3				2.4											
	Pork	Danish	0	[0-13.2]		100																
		Imported	0	[0-5.8]	1.6	91.9	6.5															
Nalidixic acid	Broiler meat	Imported	0	[0-18.5]									94.4	5.6								
	Turkey meat	Imported	2.4	[0.06-12.9]									65.9	29.3	2.4			2.4				
	Pork	Danish	0	[0-13.2]									84.6	15.4								
		Imported	0	[0-5.8]									75.8	22.6	1.6							
Colistin	Broiler meat	Imported	0	[0-18.5]							100											
	Turkey meat	Imported	2.4	[0.06-12.9]							97.6			2.4								
	Pork	Danish	0	[0-13.2]							100											
		Imported	0	[0-5.8]							96.8	3.2										
Spectinomycin	Broiler meat	Imported	0	[0-18.5]												77.8	22.2					
	Turkey meat	Imported	39.0	[24.2-55.5]												41.5	19.5	2.4		36.6		
	Pork	Danish	7.7	[0.9-25.1]												73.1	19.2			7.7		
		Imported	24.2	[14.2-36.7]												43.5	32.3		3.2	21.0		

Vertical solid lines indicate EUCAST epidemiological cut-off values except for apramycin and sulfonamide where the cut-off values were set by DANMAP. EUCAST/CLSI clinical breakpoints indicated as vertical dotted lines if different from the corresponding epidemiological cut-off values. See table AP2.2 for further details

White fields represent the range of dilutions tested. MIC values equal to or lower than the lowest concentration tested are presented as the lowest concentration. MIC values greater than the highest concentration in the range are presented as one dilution step above the range.

Table AP1.11. Distribution of MICs and resistance (%) in *Salmonella* Typhimurium from human cases reported as (n=227), domestic outbreak related (n=212), assosiated with travel abroad (n=95) and of unknown origin (n=95), Denmark

DANMAP 2010

Antimicrobial agent	Origin	% Resistant	95% Confidence interval	Distribution (%) of MICs																		
				0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	>2048
Tetracycline	Domestic sporadic	36.1	[29.9-42.7]								63.4	0.4		0.4	3.5	32.2						
	Domestic outbreak	21.2	[15.9-27.4]								78.8				1.4	19.8						
	Travel abroad reported	58.9	[48.4-68.9]								41.1			2.1	4.2	52.6						
	Unknown origin	53.7	[43.2-64.0]								45.3	1.1			4.2	49.5						
Chloramphenicol	Domestic sporadic	7.5	[4.4-11.7]								0.4	19.8	70.5	1.8		0.4	7.0					
	Domestic outbreak	3.8	[1.6-7.3]									3.8	92.5			0.9	2.8					
	Travel abroad reported	12.6	[6.7-21.0]								1.1	18.9	66.3	1.1			12.6					
	Unknown origin	13.7	[7.5-22.3]									18.9	64.2	3.2		2.1	11.6					
Florfenicol	Domestic sporadic	6.2	[3.4-10.1]								1.3	82.4	9.3	0.9	4.8	1.3						
	Domestic outbreak	3.8	[1.6-7.3]									82.5	13.7		3.8							
	Travel abroad reported	11.6	[5.9-19.8]								3.2	73.7	11.6		7.4		4.2					
	Unknown origin	10.5	[5.2-18.5]								1.1	77.9	6.3	4.2	4.2	4.2	2.1					
Ampicillin	Domestic sporadic	41.9	[35.4-48.6]							30.8	26.4	0.9				41.9						
	Domestic outbreak	77.8	[71.6-83.2]							10.8	11.3					77.8						
	Travel abroad reported	60.0	[49.4-69.9]							23.2	16.8					60.0						
	Unknown origin	57.9	[47.3-68.0]							25.3	15.8	1.1				57.9						
Ceftiofur	Domestic sporadic	0	[0-1.6]						57.7	39.6	2.6											
	Domestic outbreak	0	[0-1.7]						61.3	38.2	0.5											
	Travel abroad reported	3.2	[0.7-9.0]						62.1	33.7	1.1			3.2								
	Unknown origin	1.1	[0.03-5.7]						60.0	37.9	1.1			1.1								
Trimethoprim	Domestic sporadic	4.8	[2.4-8.5]							95.2		0.4				4.4						
	Domestic outbreak	0.9	[0.1-3.4]							98.6	0.5					0.9						
	Travel abroad reported	8.4	[3.7-15.9]							91.6						8.4						
	Unknown origin	8.4	[3.7-15.9]							90.5	1.1					8.4						
Sulfonamide	Domestic sporadic	43.6	[37.1-50.3]													55.5	0.4	0.4		0.4	43.2	
	Domestic outbreak	87.3	[82.0-91.4]													12.7					87.3	
	Travel abroad reported	64.2	[53.7-73.8]													31.6	4.2				64.2	
	Unknown origin	63.2	[52.6-72.8]													35.8	1.1				63.2	
Streptomycin	Domestic sporadic	42.7	[36.2-49.4]										48.9	8.4	1.8	4.4	13.2	23.3				
	Domestic outbreak	87.3	[82.0-91.4]										10.8	1.9		3.3	28.3	55.7				
	Travel abroad reported	58.9	[48.4-68.9]										33.7	7.4	2.1	6.3	12.6	37.9				
	Unknown origin	57.9	[47.3-68.0]										35.8	6.3	2.1	7.4	17.9	30.5				
Gentamicin	Domestic sporadic	0.9	[0.1-3.1]						96.0	2.2	0.9	0.4	0.4									
	Domestic outbreak	0.5	[0.01-2.6]						94.3	5.2		0.5										
	Travel abroad reported	3.2	[0.7-9.0]						94.7	2.1					3.2							
	Unknown origin	2.1	[0.3-7.4]						97.9						2.1							

Table AP1.11 (Continued). Distribution of MICs and resistance (%) in *Salmonella* Typhimurium from human cases reported as (n=227), domestic outbreak related (n=212), assosiated with travel abroad (n=95) and of unknown origin (n=95), Denmark

DANMAP 2010

Antimicrobial agent	Origin	% Resistant	95% Confidence interval	Distribution (%) of MICs																	
				0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048 >2048
Neomycin	Domestic sporadic	3.5	[1.5-6.8]								96.0	0.4	0.9	0.4	0.9	1.3					
	Domestic outbreak	0	[0-1.7]								100										
	Travel abroad reported	2.1	[0.3-7.4]								97.9					2.1					
	Unknown origin	4.2	[1.2-10.4]								95.8				1.1	3.2					
Apramycin	Domestic sporadic	0.9	[0.1-3.1]									97.8	0.9	0.4	0.4	0.4					
	Domestic outbreak	0	[0-1.7]									100									
	Travel abroad reported	0	[0-3.8]									98.9	1.1								
	Unknown origin	0	[0-3.8]									98.9	1.1								
Ciprofloxacin	Domestic sporadic	3.5	[1.5-6.8]	10.6	81.1	4.8		3.5													
	Domestic outbreak	3.8	[1.6-7.3]	1.4	91.0	3.8		3.8													
	Travel abroad reported	13.7	[7.5-22.3]	7.4	76.8	2.1	3.2	9.5	1.1												
	Unknown origin	14.7	[8.3-23.5]	9.5	71.6	4.2	1.1	8.4	3.2	1.1			1.1								
Nalidixic acid	Domestic sporadic	2.2	[0.7-5.1]									85.0	12.3	0.4			2.2				
	Domestic outbreak	3.8	[1.6-7.3]									61.8	34.4				3.8				
	Travel abroad reported	8.4	[3.7-15.9]									81.1	10.5				8.4				
	Unknown origin	12.6	[6.7-21.0]									70.5	15.8	1.1		1.1	11.6				
Colistin	Domestic sporadic	0.9	[0.1-3.1]							97.8	1.3	0.4		0.4							
	Domestic outbreak	0	[0-1.7]							100											
	Travel abroad reported	0	[0-3.8]							100											
	Unknown origin	1.1	[0.03-5.7]							97.9	1.1	1.1									
Spectinomycin	Domestic sporadic	9.3	[5.8-13.8]												74.0	16.7	0.4	1.3	7.5		
	Domestic outbreak	3.8	[1.6-7.3]											0.5	69.3	26.4			3.8		
	Travel abroad reported	13.7	[7.5-22.3]											1.1	71.6	13.7			13.7		
	Unknown origin	13.7	[7.5-22.3]												75.8	10.5	1.1		12.6		

Vertical solid lines indicate EUCAST epidemiological cut-off values except for apramycin, spectinomycin and sulfonamide where the cut-off values were set by DANMAP. EUCAST/CLSI clinical breakpoints indicated as vertical dotted lines if different from the corresponding epidemiological cut-off values. See table AP2.2 for further details

White fields represent the range of dilutions tested. MIC values equal to or lower than the lowest concentration tested are presented as the lowest concentration. MIC values greater than the highest concentration in the range are presented as one dilution step above the range

Table AP1.12. Distribution of MICs and resistance (%) in *Salmonella* Enteritidis from human cases reported as domestic sporadic (n=64), domestic outbreak related (n=2), associated with travel abroad (n=217) and of unknown origin (n=81), Denmark

DANMAP 2010

Antimicrobial agent	Origin	% Resistant	95% Confidence interval	Distribution (%) of MICs																	
				0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	>2048
Tetracycline	Domestic sporadic	1.6	[0.04-8.4]								93.8	4.7				1.6						
	Domestic outbreak	0	[0-84.2]								100											
	Travel abroad reported	5.1	[2.6-8.9]								89.9	5.1				5.1						
	Unknown origin	3.7	[0.8-10.4]								93.8	2.5				3.7						
Chloramphenicol	Domestic sporadic	1.6	[0.04-8.4]									35.9	62.5				1.6					
	Domestic outbreak	0	[0-84.2]									50.0	50.0									
	Travel abroad reported	0.9	[0.1-3.3]								0.5	33.2	64.5	0.9			0.9					
	Unknown origin	0	[0-4.5]									27.2	72.8									
Florfenicol	Domestic sporadic	0	[0-5.6]									85.9	14.1									
	Domestic outbreak	0	[0-84.2]									100										
	Travel abroad reported	0.5	[0.01-2.5]								0.9	93.1	4.6	0.9			0.5					
	Unknown origin	0	[0-4.5]									92.6	7.4									
Ampicillin	Domestic sporadic	3.1	[0.4-10.8]							28.1	67.2	1.6				3.1						
	Domestic outbreak	0	[0-84.2]							100												
	Travel abroad reported	7.8	[4.6-12.2]							24.0	65.9	1.8	0.5			7.8						
	Unknown origin	3.7	[0.8-10.4]							25.9	70.4					3.7						
Ceftiofur	Domestic sporadic	0	[0-5.6]						70.3	29.7												
	Domestic outbreak	0	[0-84.2]						100													
	Travel abroad reported	0	[0-1.7]						63.6	35.9	0.5											
	Unknown origin	0	[0-4.5]						70.4	29.6												
Trimethoprim	Domestic sporadic	1.6	[0.04-8.4]							98.4						1.6						
	Domestic outbreak	0	[0-84.2]							100												
	Travel abroad reported	2.3	[0.8-5.3]							97.7						2.3						
	Unknown origin	0	[0-4.5]							100												
Sulfonamide	Domestic sporadic	1.6	[0.04-8.4]													93.8	4.7				1.6	
	Domestic outbreak	0	[0-84.2]													100						
	Travel abroad reported	2.3	[0.8-5.3]													92.2	5.5				2.3	
	Unknown origin	0	[0-4.5]													95.1	4.9					
Streptomycin	Domestic sporadic	1.6	[0.04-8.4]										96.9	1.6			1.6					
	Domestic outbreak	0	[0-84.2]										100									
	Travel abroad reported	1.8	[0.5-4.7]										97.2	0.9			0.5	1.4				
	Unknown origin	1.2	[0.03-6.7]										97.5	1.2				1.2				

Table AP1.12 (Continued). Distribution of MICs and resistance (%) in *Salmonella* Enteritidis from human cases reported as domestic sporadic (n=64), domestic outbreak related (n=2), associated with travel abroad (n=217) and of unknown origin (n=81), Denmark

DANMAP 2010

Antimicrobial agent	Origin	% Resistant	95% Confidence interval	Distribution (%) of MICs																
				0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	>2048
Gentamicin	Domestic sporadic	0	[0-5.6]						98.4	1.6												
	Domestic outbreak	0	[0-84.2]						100													
	Travel abroad reported	0.5	[0.01-2.5]						99.5				0.5									
	Unknown origin	0	[0-4.5]						100													
Neomycin	Domestic sporadic	0	[0-5.6]								100											
	Domestic outbreak	0	[0-84.2]								100											
	Travel abroad reported	0	[0-1.7]								99.5	0.5										
	Unknown origin	0	[0-4.5]								100											
Apramycin	Domestic sporadic	0	[0-5.6]									100										
	Domestic outbreak	0	[0-84.2]									100										
	Travel abroad reported	0	[0-1.7]									100										
	Unknown origin	0	[0-4.5]									98.8	1.2									
Ciprofloxacin	Domestic sporadic	7.8	[2.6-17.3]	9.4	82.8			4.7	3.1													
	Domestic outbreak	100	[15.8-100.0]					100														
	Travel abroad reported	20.7	[15.5-26.7]	7.8	70.5	0.9	2.8	14.3	3.2	0.5												
	Unknown origin	21.0	[12.7-31.5]	8.6	69.1	1.2	2.5	16.0	2.5													
Nalidixic acid	Domestic sporadic	7.8	[2.6-17.3]									89.1	3.1				7.8					
	Domestic outbreak	100	[15.8-100.0]														100					
	Travel abroad reported	19.4	[14.3-25.2]									75.6	3.7	1.4			19.4					
	Unknown origin	21.0	[12.7-31.5]									79.0			1.2		19.8					
Colistin	Domestic sporadic	15.6	[7.8-26.9]							59.4	25.0	14.1	1.6									
	Domestic outbreak	0	[0-84.2]							100												
	Travel abroad reported	29.0	[23.1-35.6]							47.9	23.0	21.7	6.9	0.5								
	Unknown origin	16.0	[8.8-25.9]							55.6	28.4	13.6	2.5									
Spectinomycin	Domestic sporadic	0	[0-5.6]											3.1	87.5	9.4						
	Domestic outbreak	0	[0-84.2]											50.0	50.0							
	Travel abroad reported	0.5	[0.01-2.5]											9.7	88.5	1.4			0.5			
	Unknown origin	1.2	[0.03-6.7]											14.8	81.5	2.5		1.2				

Vertical solid lines indicate EUCAST epidemiological cut-off values except for apramycin, spectinomycin and sulfonamide where the cut-off values were set by DANMAP. EUCAST/CLSI clinical breakpoints indicated as vertical dotted lines if different from the corresponding epidemiological cut-off values. See table AP2.2 for further details

White fields represent the range of dilutions tested. MIC values equal to or lower than the lowest concentration tested are presented as the lowest concentration. MIC values greater than the highest concentration in the range are presented as one dilution step above the range

Table AP1.13. Distribution of MICs and resistance (%) in *Campylobacter coli* from pigs (n=103), Denmark

DANMAP 2010

Antimicrobial agent	% Resistant	95% Confidence interval	Distribution (%) of MICs												
			0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	>128
Tetracycline	11.7	[6.2-19.5]			52.4	24.3	9.7	1.9	2.9	1.0	1.0	6.8			
Chloramphenicol	0	[0-3.5]						20.4	59.2	18.4	1.9				
Erythromycin	15.5	[9.1-24.0]				23.3	24.3	26.2	10.7				15.5		
Streptomycin	63.1	[53.0-72.4]					28.2	8.7			3.9	59.2			
Gentamicin	0	[0-3.5]		9.7	58.3	32.0									
Ciprofloxacin	7.8	[3.4-14.7]	28.2	43.7	20.4					7.8					
Nalidixic acid	7.8	[3.4-14.7]						7.8	35.9	33.0	15.5		1.9	5.8	

Vertical solid lines indicate EUCAST epidemiological cut-off values. EUCAST/CLSI clinical breakpoints indicated as vertical dotted lines if different from the corresponding epidemiological cut-off values. See table AP2.2 for further details
White fields represent the range of dilutions tested. MIC values equal to or lower than the lowest concentration tested are presented as the lowest concentration. MIC values greater than the highest concentration in the range are presented as one dilution step above the range

Table AP1.14. Distribution of MICs and resistance (%) in *Campylobacter jejuni* from broilers (n=41) and cattle (n=98), Denmark

DANMAP 2010

Antimicrobial agent	Animal species	% Resistant	95% Confidence interval	Distribution (%) of MICs												
				0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	>128
Tetracycline	Broilers	17.1	[7.2-32.1]			61.0	17.1	4.9				2.4	14.6			
	Cattle	6.1	[2.3-12.9]			59.2	32.7	1.0	1.0				6.1			
Chloramphenicol	Broilers	0	[0-8.6]						58.5	36.6	4.9					
	Cattle	0	[0-3.7]						76.5	22.4	1.0					
Erythromycin	Broilers	0	[0-8.6]				46.3	26.8	19.5	7.3						
	Cattle	0	[0-3.7]				33.7	48.0	17.3	1.0						
Streptomycin	Broilers	2.4	[0.06-12.9]					97.6					2.4			
	Cattle	1.0	[0.03-5.6]					98.0	1.0				1.0			
Gentamicin	Broilers	0	[0-8.6]		46.3	46.3	4.9	2.4								
	Cattle	0	[0-3.7]		42.9	48.0	9.2									
Ciprofloxacin	Broilers	19.5	[8.8-34.9]	12.2	46.3	19.5	2.4				19.5					
	Cattle	20.4	[12.9-29.7]	10.2	59.2	9.2	1.0				20.4					
Nalidixic acid	Broilers	17.1	[7.2-32.1]						7.3	53.7	17.1	4.9			17.1	
	Cattle	20.4	[12.9-29.7]						5.1	59.2	13.3	2.0			20.4	

Vertical solid lines indicate EUCAST epidemiological cut-off values. EUCAST/CLSI clinical breakpoints indicated as vertical dotted lines if different from the corresponding epidemiological cut-off values. See table AP2.2 for further details
White fields represent the range of dilutions tested. MIC values equal to or lower than the lowest concentration tested are presented as the lowest concentration. MIC values greater than the highest concentration in the range are presented as one dilution step above the range

Table AP1.15. Distribution of MICs and resistance (%) in *Campylobacter coli* from broiler meat (Danish n=20; imported n=27), Denmark

DANMAP 2010

Antimicrobial agent	Origin	% Resistant	95% Confidence interval	Distribution (%) of MICs											
				0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	>64
Tetracycline	Danish	35.0	[15.4-59.2]			45.0	15.0	5.0				35.0			
	Imported	81.5	[61.9-93.7]			14.8	3.7					81.5			
Chloramphenicol	Danish	0	[0-16.8]						45.0	40.0	15.0				
	Imported	0	[0-12.8]						14.8	59.3	22.2	3.7			
Erythromycin	Danish	0	[0-16.8]				40.0	50.0	10.0						
	Imported	14.8	[4.2-33.7]				25.9	18.5	29.6	11.1			14.8		
Streptomycin	Danish	20.0	[5.7-43.7]					70.0	10.0			20.0			
	Imported	0	[0-12.8]					85.2	14.8						
Gentamicin	Danish	0	[0-16.8]		50.0	35.0	15.0								
	Imported	0	[0-12.8]		18.5	51.9	29.6								
Ciprofloxacin	Danish	0	[0-16.8]	5.0	60.0	25.0	10.0								
	Imported	85.2	[66.3-95.8]		7.4	7.4				85.2					
Nalidixic acid	Danish	0	[0-16.8]						10.0	70.0	15.0		5.0		
	Imported	85.2	[66.3-95.8]							7.4	7.4			85.2	

Vertical solid lines indicate EUCAST epidemiological cut-off values. EUCAST/CLSI clinical breakpoints indicated as vertical dotted lines if different from the corresponding epidemiological cut-off values. See table AP2.2 for further details

White fields represent the range of dilutions tested. MIC values equal to or lower than the lowest concentration tested are presented as the lowest concentration. MIC values greater than the highest concentration in the range are presented as one dilution step above the range

Table AP1.16. Distribution of MICs and resistance (%) in *Campylobacter jejuni* from broiler meat (Danish n=52; imported n=68), Denmark

DANMAP 2010

Antimicrobial agent	Origin	% Resistant	95% Confidence interval	Distribution (%) of MICs											
				0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	>64
Tetracycline	Danish	11.5	[4.4-23.4]			59.6	23.1	1.9	3.8			11.5			
	Imported	41.2	[29.4-53.8]			29.4	26.5	1.5	1.5			41.2			
Chloramphenicol	Danish	0	[0-6.8]						73.1	21.2	5.8				
	Imported	0	[0-5.3]						41.2	38.2	11.8	8.8			
Erythromycin	Danish	1.9	[0.05-10.3]				34.6	34.6	25.0	3.8	1.9				
	Imported	4.4	[0.9-12.4]				17.6	52.9	17.6	7.4			4.4		
Streptomycin	Danish	1.9	[0.05-10.3]					98.1				1.9			
	Imported	0	[0-5.3]					94.1	5.9						
Gentamicin	Danish	0	[0-6.8]		48.1	50.0	1.9								
	Imported	0	[0-5.3]		33.8	52.9	13.2								
Ciprofloxacin	Danish	17.3	[8.2-30.3]	3.8	63.5	11.5	1.9	1.9	1.9	15.4					
	Imported	50.0	[37.6-62.4]	7.4	22.1	13.2	4.4	2.9		50.0					
Nalidixic acid	Danish	13.5	[5.6-25.8]						13.5	57.7	13.5	1.9	1.9	11.5	
	Imported	50.0	[37.6-62.4]						4.4	33.8	5.9	5.9		50.0	

Vertical solid lines indicate EUCAST epidemiological cut-off values. EUCAST/CLSI clinical breakpoints indicated as vertical dotted lines if different from the corresponding epidemiological cut-off values. See table AP2.2 for further details

White fields represent the range of dilutions tested. MIC values equal to or lower than the lowest concentration tested are presented as the lowest concentration. MIC values greater than the highest concentration in the range are presented as one dilution step above the range

Table AP1.17. Distribution of MICs and resistance (%) in *Campylobacter jejuni* from human cases reported as domestic sporadic (n=52), assosiated with travel abroad (n=46) and of unknown origin (n=43), Denmark

DANMAP 2010

Antimicrobial agent	Origin	% Resistant	95% Confidence interval	Distribution (%) of MICs												
				0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	>128
Tetracycline	Domestically acquired	13.5	[5.6-25.8]			69.2	15.4	1.9					13.5			
	Travel abroad reported	56.5	[41.1-71.1]			26.1	13.0	2.2	2.2				56.5			
	Unknown origin	20.9	[10.0-36.0]			46.5	23.3	9.3					20.9			
Chloramphenicol	Domestically acquired	0	[0-6.8]						73.1	26.9						
	Travel abroad reported	0	[0-7.7]						45.7	30.4	21.7	2.2				
	Unknown origin	0	[0-8.2]						67.4	25.6	7.0					
Erythromycin	Domestically acquired	0	[0-6.8]				44.2	48.1	7.7							
	Travel abroad reported	0	[0-7.7]				43.5	43.5	10.9	2.2						
	Unknown origin	0	[0-8.2]				41.9	46.5	7.0	4.7						
Streptomycin	Domestically acquired	1.9	[0.05-10.3]					98.1					1.9			
	Travel abroad reported	2.2	[0.06-11.5]					95.7	2.2		2.2					
	Unknown origin	2.3	[0.06-12.3]					95.3	2.3	2.3						
Gentamicin	Domestically acquired	0	[0-6.8]		69.2	28.8		1.9								
	Travel abroad reported	0	[0-7.7]		63.0	34.8	2.2									
	Unknown origin	2.3	[0.06-12.3]		46.5	48.8	2.3						2.3			
Ciprofloxacin	Domestically acquired	25.0	[14.0-38.9]	19.2	48.1	5.8	1.9			1.9	23.1					
	Travel abroad reported	80.4	[66.1-90.6]	6.5	10.9		2.2				80.4					
	Unknown origin	41.9	[27.0-57.9]	9.3	32.6	11.6	2.3	2.3		2.3	39.5					

Vertical solid lines indicate EUCAST epidemiological cut-off values. EUCAST/CLSI clinical breakpoints indicated as vertical dotted lines if different from the corresponding epidemiological cut-off values. See table AP2.2 for further details

White fields represent the range of dilutions tested. MIC values equal to or lower than the lowest concentration tested are presented as the lowest concentration. MIC values greater than the highest concentration in the range are presented as one dilution step above the range

Figure AP1.2. Resistance (%) to tetracycline among *Enterococcus faecium* and *Enterococcus faecalis* from pigs and the consumption of tetracyclines in pigs, Denmark

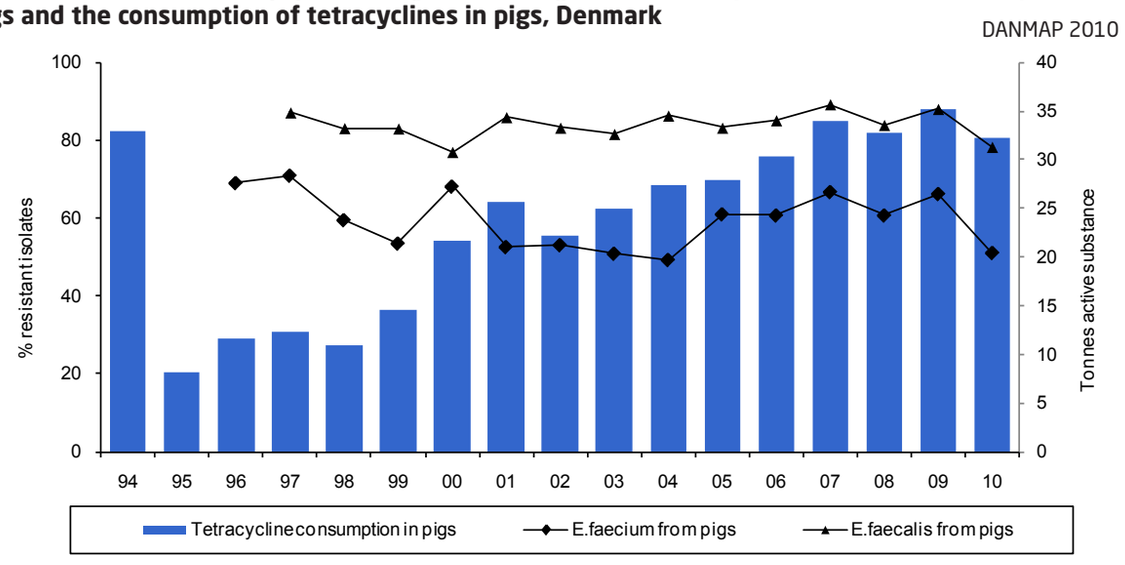


Figure AP1.3. Resistance (%) to erythromycin among *Enterococcus faecium* and *Enterococcus faecalis* from pigs and the the consumption of macrolides in pigs, Denmark

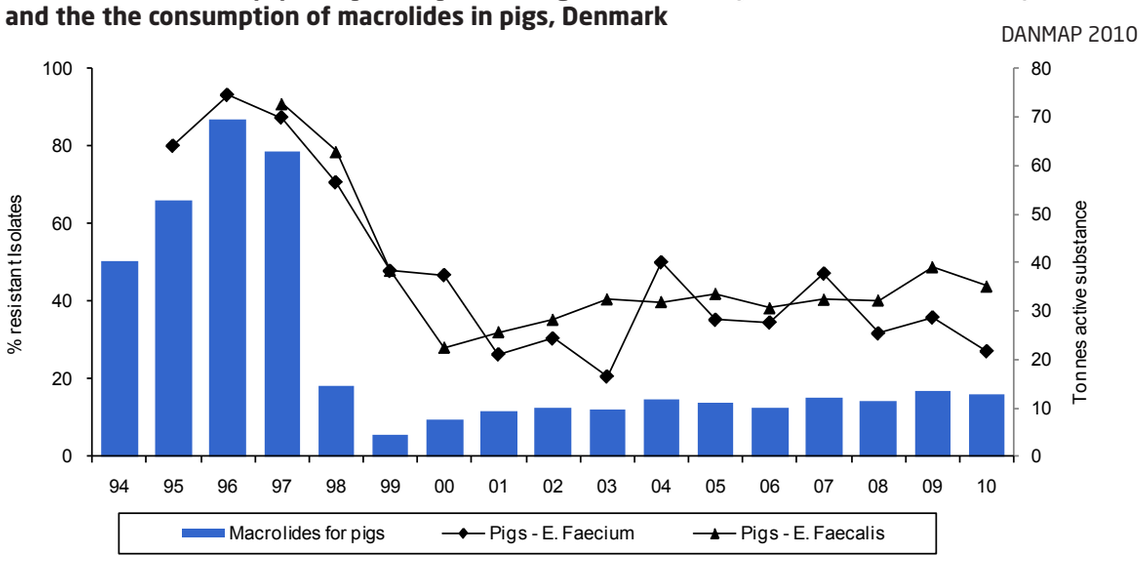


Figure AP1.4. Resistance (%) to streptogramins in *Enterococcus faecium* from broilers and the consumption of virginiamycin, Denmark

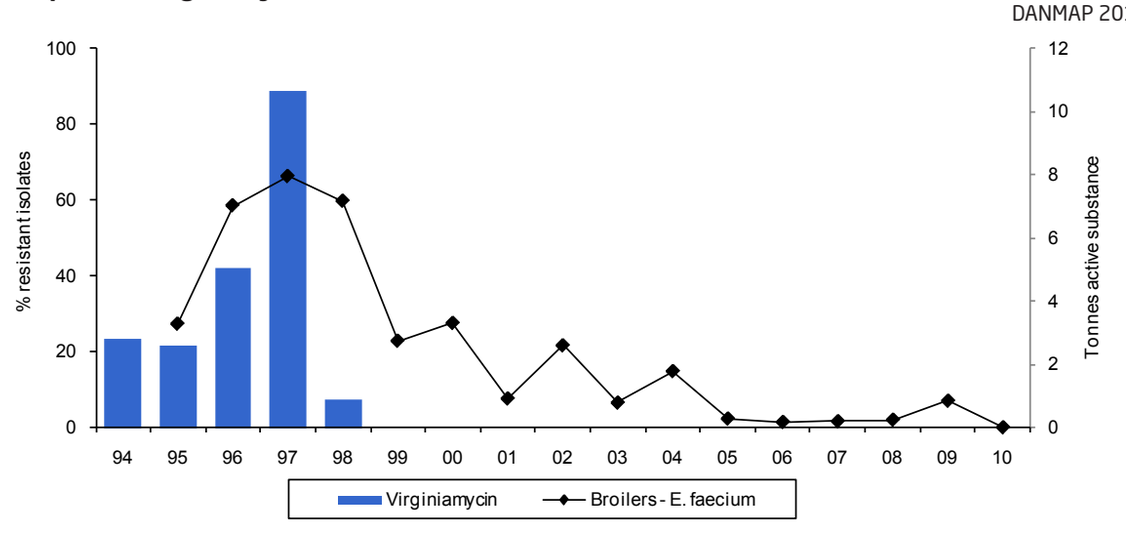


Figure AP1.5. Resistance (%) to avoparcin in *Enterococcus faecium* and *Enterococcus faecalis* from broilers and the consumption of avoparcin, Denmark

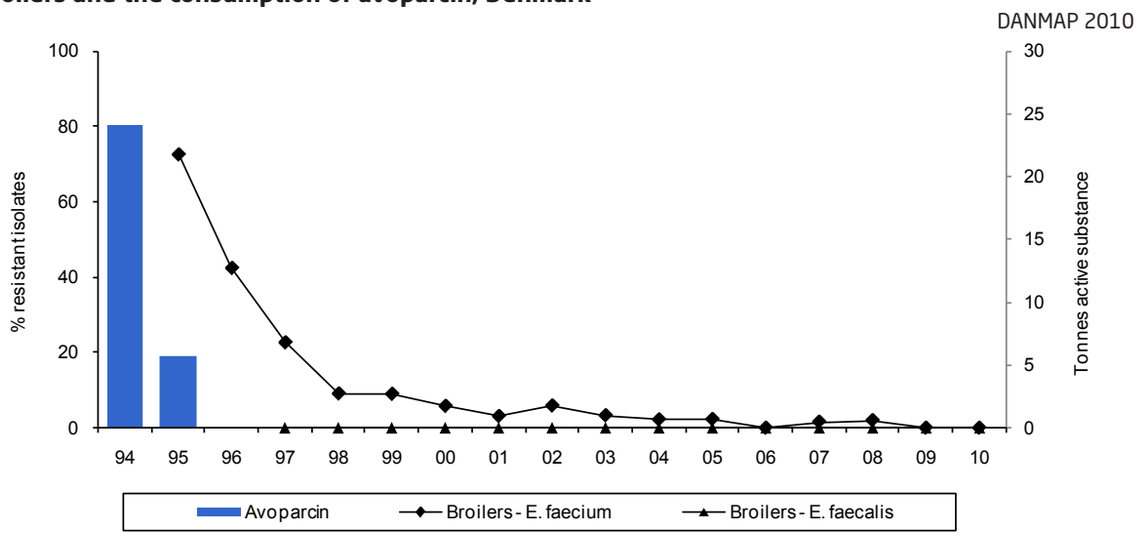


Figure AP1.6. Resistance (%) to streptogramins in *Enterococcus faecium* from pigs and the comsumption of virginiamycin, Denmark

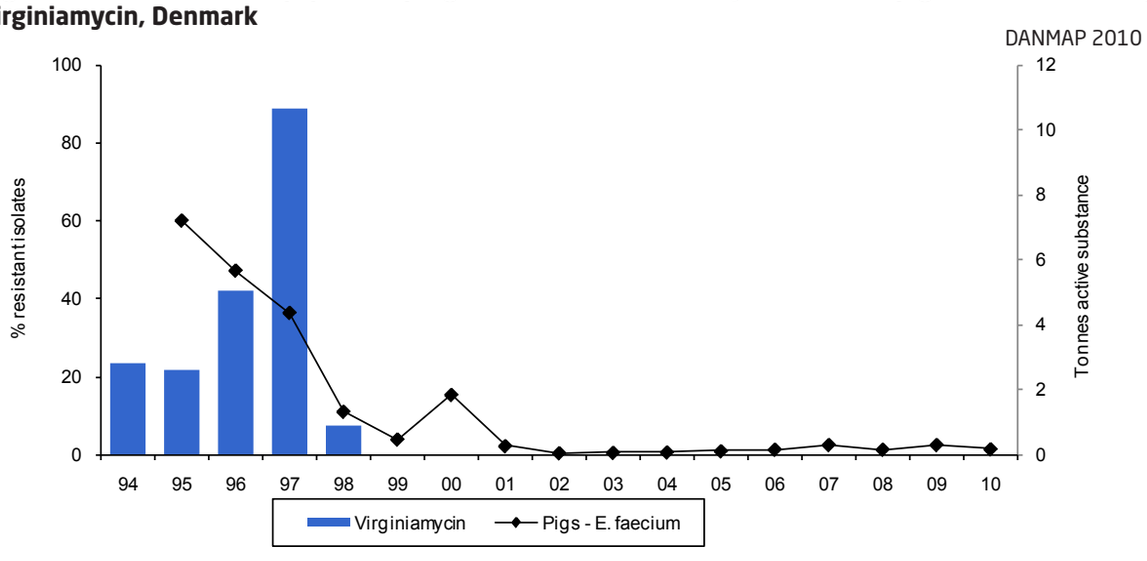


Figure AP1.7. Resistance (%) to avoparcin in *Enterococcus faecium* and *Enterococcus faecalis* from pigs and the consumption of avoparcin, Denmark

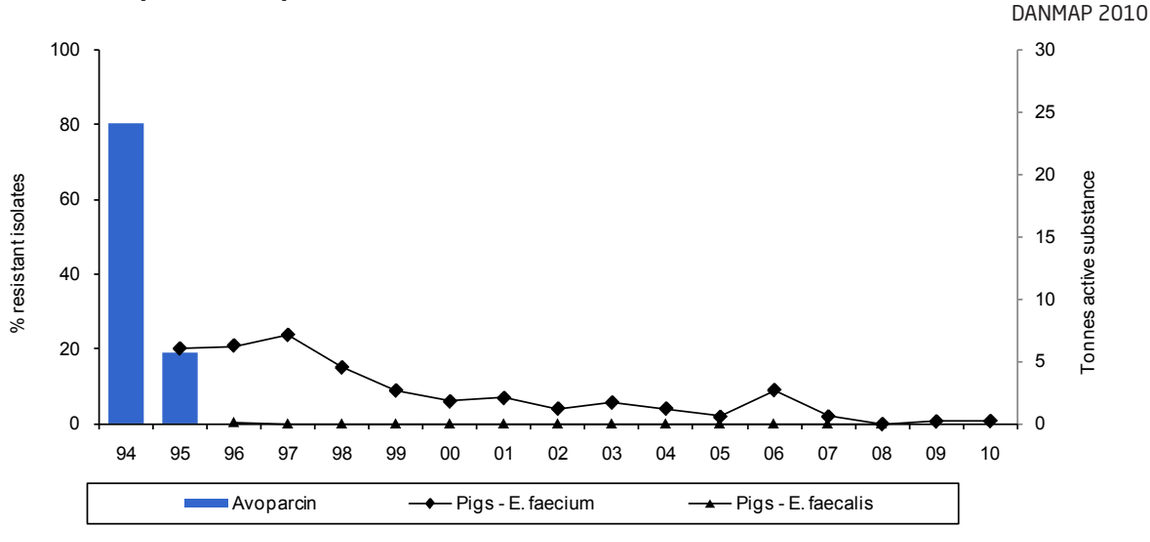


Table AP1.18. Distribution of MICs and resistance (%) in *Enterococcus faecium* from broilers (n=119) and pigs (n=133), Denmark DANMAP 2010

Antimicrobial agent	Animal species	% Resistant	95% Confidence interval	Distribution (%) of MICs																		
				0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	4096	>4096
Tetracycline	Broilers	5.9	[2.4-11.7]							93.3	0.8			0.8	0.8	4.2							
	Pigs	51.1	[42.3-59.9]							48.1	0.8		0.8		2.3	48.1							
Tigecycline	Broilers	0	[0-3.1]		3.4	93.3	3.4																
	Pigs	0	[0-2.7]		6.0	78.9	15.0																
Chloramphenicol	Broilers	0	[0-3.1]								0.8	31.9	66.4	0.8									
	Pigs	0	[0-2.7]								3.0	48.9	46.6	1.5									
Penicillin	Broilers	0.8	[0.02-4.6]								56.3	18.5	13.4	10.9	0.8								
	Pigs	3.0	[0.8-7.5]								26.3	20.3	6.0	44.4	3.0								
Ampicillin	Broilers	0	[0-3.1]								89.1	10.9											
	Pigs	2.3	[0.5-6.5]								51.9	45.9	2.3										
Erythromycin	Broilers	26.1	[18.4-34.9]						11.8	18.5	13.4	30.3	2.5	6.7	10.1	6.7							
	Pigs	27.1	[19.7-35.5]						12.0	6.0	34.6	20.3	2.3			24.8							
Quinupristin/ dalfopristin	Broilers	0	[0-3.1]					0.8	50.4	9.2	37.0	2.5											
	Pigs	1.5	[0.2-5.3]					0.8	21.1	5.3	52.6	18.8	0.8	0.8									
Streptomycin	Broilers	0.8	[0.02-4.6]													97.5	1.7					0.8	
	Pigs	34.6	[26.6-43.3]													63.2	2.3	1.5	0.8	3.0	14.3	15.0	
Gentamicin	Broilers	0	[0-3.1]											95.0	5.0								
	Pigs	0	[0-2.7]											97.7	2.3								
Kanamycin	Broilers	0	[0-3.1]														36.1	41.2	20.2	2.5			
	Pigs	23.3	[16.4-31.4]														25.6	31.6	15.8	3.8		23.3	
Vancomycin	Broilers	0	[0-3.1]							60.5	37.8	1.7											
	Pigs	0.8	[0.02-4.1]							91.7	6.0	1.5				0.8							
Linezolid	Broilers	0	[0-3.1]							3.4	84.0	12.6											
	Pigs	0	[0-2.7]							12.8	76.7	10.5											
Avilamycin	Broilers	0	[0-3.1]									97.5	0.8	1.7									
	Pigs	0	[0-2.7]									91.7	8.3										
Salinomycin	Broilers	52.9	[43.6-62.2]								4.2	42.9	52.9										
	Pigs	0	[0-2.7]								99.2	0.8											

Vertical solid lines indicate EUCAST epidemiological cut-off values except for ciprofloxacin, kanamycin, quinupristin/dalfopristin and salinomycin where the cut-off values were set by DANMAP. EUCAST/CLSI clinical breakpoints indicated as vertical dotted lines if different from the corresponding epidemiological cut-off values. See table AP2.2 for further details
White fields represent the range of dilutions tested. MIC values equal to or lower than the lowest concentration tested are presented as the lowest concentration. MIC values greater than the highest concentration in the range are presented as one dilution step above the range

Table AP1.19. Distribution of MICs and resistance (%) in *Enterococcus faecalis* from broilers (n=112) and pigs (n=157), Denmark

Antimicrobial agent	Animal species	% Resistant	95% Confidence interval	Distribution (%) of MICs																		
				0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	4096	>4096
Tetracycline	Broilers	25.9	[18.1-35.0]							73.2	0.9				16.1	9.8							
	Pigs	78.3	[71.1-84.5]							21.7				0.6	9.6	68.2							
Tigecycline	Broilers	0	[0-3.2]		2.7	28.6	68.8																
	Pigs	0	[0-2.3]		8.3	47.1	38.9	5.7															
Chloramphenicol	Broilers	0	[0-3.2]									44.6	55.4										
	Pigs	15.9	[10.6-22.6]									38.2	45.9			5.7	10.2						
Penicillin	Broilers	0	[0-3.2]								57.1	42.9											
	Pigs	0	[0-2.3]								25.5	74.5											
Ampicillin	Broilers	0	[0-3.2]								100												
	Pigs	0	[0-2.3]								99.4	0.6											
Erythromycin	Broilers	25.0	[17.3-34.1]						18.8	44.6	11.6		0.9	2.7	3.6	17.9							
	Pigs	43.9	[36.0-52.1]						16.6	35.0	4.5					43.9							
Quinupristin/ dalfopristin	Broilers	93.8	[87.5-97.5]						0.9			5.4	84.8	8.9									
	Pigs	98.7	[95.5-99.8]								0.6	0.6	68.2	26.8	3.8								
Streptomycin	Broilers	3.6	[1.0-8.9]													2.7	92.0	1.8				3.6	
	Pigs	28.0	[21.2-35.7]													2.5	68.2	1.3		0.6	1.9	25.5	
Gentamicin	Broilers	0.9	[0.02-4.9]											93.8	5.4						0.9		
	Pigs	11.5	[6.9-17.5]											75.8	12.7			1.3	2.5	2.5	5.1		
Kanamycin	Broilers	0.9	[0.02-4.9]														99.1					0.9	
	Pigs	21.0	[14.9-28.2]														79.0					21.0	
Vancomycin	Broilers	0	[0-3.2]							40.2	52.7	7.1											
	Pigs	0	[0-2.3]							22.3	71.3	6.4											
Linezolid	Broilers	0	[0-3.2]							47.3	52.7												
	Pigs	0	[0-2.3]							49.0	51.0												
Avilamycin	Broilers	0	[0-3.2]									100											
	Pigs	0	[0-2.3]									100											
Salinomycin	Broilers	0	[0-3.2]								82.1	17.9											
	Pigs	0	[0-2.3]								100												

Vertical solid lines indicate EUCAST epidemiological cut-off values except for ciprofloxacin, kanamycin and salinomycin where the cut-off values were set by DANMAP. EUCAST/CLSI clinical breakpoints indicated as vertical dotted lines if different from the corresponding epidemiological cut-off values. See table AP2.2 for further details

White fields represent the range of dilutions tested. MIC values equal to or lower than the lowest concentration tested are presented as the lowest concentration. MIC values greater than the highest concentration in the range are presented as one dilution step above the range

Table AP1.20. Distribution of MICs and and resistance (%) in *Enterococcus faecium* from broiler meat (Danish n=145; imported n=107), Danish beef (n=20) and Danish pork (n=29), Denmark

DANMAP 2010

Antimicrobial agent	Food type	Origin	% Resistant	95% Confidence interval	Distribution (%) of MICs																		
					0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	4096	>4096
Tetracycline	Broiler meat	Danish	10.3	[5.9-16.5]							86.9	2.1	0.7		0.7	2.1	7.6							
		Imported	43.0	[33.5-52.9]							56.1		0.9		0.9	3.7	38.3							
	Beef	Danish	10.0	[1.2-31.7]							90.0						10.0							
	Pork	Danish	17.2	[5.8-35.8]							79.3	3.4					17.2							
Tigecycline	Broiler meat	Danish	0	[0-2.5]		11.7	80.0	8.3																
		Imported	0	[0-3.4]		2.8	67.3	29.0	0.9															
	Beef	Danish	0	[0-16.8]			95.0	5.0																
	Pork	Danish	0	[0-11.9]		6.9	93.1																	
Chloramphenicol	Broiler meat	Danish	0	[0-2.5]								2.8	55.9	37.2	2.8	1.4								
		Imported	0	[0-3.4]								0.9	17.8	59.8	16.8	4.7								
	Beef	Danish	0	[0-16.8]									40.0	60.0										
	Pork	Danish	0	[0-11.9]								3.4	48.3	41.4	6.9									
Penicillin	Broiler meat	Danish	1.4	[0.2-4.9]								62.1	24.1	9.0	3.4	1.4								
		Imported	26.2	[18.1-35.6]								35.5	15.0	14.0	9.3	6.5	19.6							
	Beef	Danish	0	[0-16.8]								50.0	45.0	5.0										
	Pork	Danish	3.4	[0.09-17.8]								44.8	44.8		6.9	3.4								
Ampicillin	Broiler meat	Danish	1.4	[0.2-4.9]								93.1	5.5	1.4										
		Imported	25.2	[17.3-34.6]								63.6	11.2	5.6	2.8	3.7	13.1							
	Beef	Danish	0	[0-16.8]								100												
	Pork	Danish	0	[0-11.9]								89.7	10.3											
Erythromycin	Broiler meat	Danish	21.4	[15.0-29.0]						19.3	15.9	18.6	24.8	4.1	6.9	3.4	6.9							
		Imported	62.6	[52.7-71.8]						15.0	10.3	8.4	3.7	3.7	3.7	0.9	54.2							
	Beef	Danish	5.0	[0.1-24.9]						20.0	10.0	50.0	15.0	5.0										
	Pork	Danish	31.0	[15.3-50.8]						17.2	6.9	10.3	34.5	20.7	3.4		6.9							
Quinupristin/ dalfopristin	Broiler meat	Danish	1.4	[0.2-4.9]					0.7	44.8	14.5	36.6	2.1	1.4										
		Imported	9.3	[4.6-16.5]						11.2	18.7	43.9	16.8	8.4	0.9									
	Beef	Danish	0	[0-16.8]					5.0	50.0	5.0	40.0												
	Pork	Danish	0	[0-11.9]					3.4	10.3	10.3	75.9												
Streptomycin	Broiler meat	Danish	2.8	[0.8-6.9]													93.8	3.4				0.7	2.1	
		Imported	37.4	[28.2-47.3]													55.1	7.5		0.9		5.6	30.8	
	Beef	Danish	0	[0-16.8]													90.0	10.0						
	Pork	Danish	6.9	[0.8-22.8]													86.2	6.9			3.4		3.4	

Table AP1.20 (Continued). Distribution of MICs and and resistance (%) in *Enterococcus faecium* from broiler meat (Danish n=145; imported n=107), Danish beef (n=20) and Danish pork (n=29), Denmark

DANMAP 2010

Antimicrobial agent	Food type	Origin	% Resistant	95% Confidence interval	Distribution (%) of MICs																	
					0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	4096	>4096
Gentamicin	Broiler meat	Danish	0	[0-2.5]											98.6	1.4								
		Imported	0	[0-3.4]											92.5	7.5								
	Beef	Danish	0	[0-16.8]											100									
	Pork	Danish	0	[0-11.9]											100									
Kanamycin	Broiler meat	Danish	1.4	[0.2-4.9]														39.3	43.4	11.7	4.1		1.4	
		Imported	19.6	[12.6-28.4]														18.7	36.4	20.6	4.7		19.6	
	Beef	Danish	0	[0-16.8]														50.0	35.0	5.0	10.0			
	Pork	Danish	3.4	[0.09-17.8]														31.0	24.1	41.4			3.4	
Ciprofloxacin	Broiler meat	Danish	0	[0-2.5]						5.5	53.8	22.1	15.9	2.1	0.7									
		Imported	0	[0-3.4]						1.9	16.8	35.5	40.2	5.6										
	Beef	Danish	0	[0-16.8]						15.0	30.0	25.0	30.0											
	Pork	Danish	0	[0-11.9]						41.4	44.8	6.9	6.9											
Vancomycin	Broiler meat	Danish	0.7	[0.02-3.8]							63.4	31.7	4.1				0.7							
		Imported	0	[0-3.4]							84.1	12.1	3.7											
	Beef	Danish	0	[0-16.8]							90.0	5.0	5.0											
	Pork	Danish	0	[0-11.9]							96.6	3.4												
Teicoplanin	Broiler meat	Danish	0.7	[0.02-3.8]					91.7	6.9	0.7						0.7							
		Imported	0	[0-3.4]					93.5	6.5														
	Beef	Danish	0	[0-16.8]					45.0	55.0														
	Pork	Danish	0	[0-11.9]					37.9	62.1														
Linezolid	Broiler meat	Danish	0	[0-2.5]							6.2	82.1	11.7											
		Imported	0	[0-3.4]							9.3	75.7	15.0											
	Beef	Danish	0	[0-16.8]							5.0	80.0	15.0											
	Pork	Danish	0	[0-11.9]						3.4		75.9	20.7											
Salinomycin	Broiler meat	Danish	36.6	[28.7-44.9]								11.0	52.4	36.6										
		Imported	10.3	[5.2-17.7]								24.3	65.4	10.3										
	Beef	Danish	0	[0-16.8]								95.0	5.0											
	Pork	Danish	0	[0-11.9]								93.1	6.9											

Vertical solid lines indicate EUCAST epidemiological cut-off values except for ciprofloxacin, kanamycin, quinupristin/dalfopristin and salinomycin where the cut-off values were set by DANMAP. EUCAST/CLSI clinical breakpoints indicated as vertical dotted lines if different from the corresponding epidemiological cut-off values. See table AP2.2 for further details
White fields represent the range of dilutions tested. MIC values equal to or lower than the lowest concentration tested are presented as the lowest concentration. MIC values greater than the highest concentration in the range are presented as one dilution step above the range

Table AP1.21. Distribution of MICs and resistance (%) in *Enterococcus faecalis* from broiler meat (Danish n=59; imported n=104), beef (Danish n=27; imported n=36) and pork (Danish n=84; imported n=91), Denmark

DANMAP 2010

Antimicrobial agent	Food type	Origin	% Resistant	95% Confidence interval	Distribution (%) of MICs																	
					0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	4096	>4096
Tetracycline	Broiler meat	Danish	45.8	[32.7-59.2]							52.5	1.7				13.6	32.2							
		Imported	54.8	[44.7-64.6]							45.2				1.0	11.5	42.3							
	Beef	Danish	22.2	[8.6-42.3]							77.8					11.1	11.1							
		Imported	19.4	[8.2-36.0]							80.6					5.6	13.9							
	Pork	Danish	13.1	[6.7-22.2]							86.9					1.2	11.9							
		Imported	34.1	[24.5-44.7]							65.9					9.9	24.2							
Tigecycline	Broiler meat	Danish	0	[0-6.1]		6.8	47.5	35.6	10.2															
		Imported	0	[0-3.5]	1.0	10.6	35.6	50.0	2.9															
	Beef	Danish	0	[0-12.8]	3.7	18.5	48.1	25.9	3.7															
		Imported	0	[0-9.7]	2.8	8.3	55.6	30.6	2.8															
	Pork	Danish	0	[0-4.3]	1.2	20.2	50.0	27.4	1.2															
		Imported	0	[0-4.0]		14.3	41.8	37.4	6.6															
Chloramphenicol	Broiler meat	Danish	1.7	[0.04-9.1]									50.8	47.5			1.7							
		Imported	4.8	[1.6-10.9]								1.0	35.6	58.7			4.8							
	Beef	Danish	0	[0-12.8]								3.7	74.1	22.2										
		Imported	2.8	[0.07-14.5]									52.8	44.4			2.8							
	Pork	Danish	1.2	[0.03-6.5]									58.3	39.3		1.2	1.2							
		Imported	3.3	[0.7-9.3]									58.2	38.5			1.1	2.2						
Penicillin	Broiler meat	Danish	1.7	[0.04-9.1]								76.3	20.3	1.7			1.7							
		Imported	0	[0-3.5]								73.1	26.9											
	Beef	Danish	0	[0-12.8]								74.1	25.9											
		Imported	0	[0-9.7]								83.3	16.7											
	Pork	Danish	0	[0-4.3]								90.5	9.5											
		Imported	0	[0-4.0]								83.5	16.5											
Ampicillin	Broiler meat	Danish	1.7	[0.04-9.1]								96.6	1.7				1.7							
		Imported	0	[0-3.5]								100												
	Beef	Danish	0	[0-12.8]								96.3	3.7											
		Imported	0	[0-9.7]								97.2	2.8											
	Pork	Danish	0	[0-4.3]								97.6	2.4											
		Imported	0	[0-4.0]								98.9	1.1											
Erythromycin	Broiler meat	Danish	16.9	[8.4-29.0]						39.0	35.6	6.8	1.7		6.8	1.7	8.5							
		Imported	39.4	[30.0-49.5]						28.8	14.4	17.3			1.0		38.5							
	Beef	Danish	0	[0-12.8]						66.7	25.9	7.4												
		Imported	2.8	[0.07-14.5]						47.2	22.2	27.8					2.8							
	Pork	Danish	1.2	[0.03-6.5]						61.9	26.2	10.7					1.2							
		Imported	5.5	[1.8-12.4]						53.8	26.4	14.3					5.5							
Quinupristin/ dalfopristin	Broiler meat	Danish	98.3	[90.9-100.0]									1.7	91.5	6.8									
		Imported	97.1	[91.8-99.4]						1.0			1.9	76.9	14.4	5.8								
	Beef	Danish	66.7	[46.0-83.5]								3.7	29.6	63.0	3.7									
		Imported	80.6	[64.0-91.8]						2.8		5.6	11.1	72.2	5.6	2.8								
	Pork	Danish	82.1	[72.3-89.6]						7.1	1.2	1.2	8.3	82.1										
		Imported	69.2	[58.7-78.5]						8.8	5.5		16.5	65.9	3.3									

Streptomycin	Broiler meat	Danish	8.5	[2.8-18.7]														76.3	15.3				8.5	
		Imported	24.0	[16.2-33.4]													1.9	58.7	14.4	1.0			24.0	
	Beef	Danish	3.7	[0.09-19.0]													14.8	77.8	3.7				3.7	
		Imported	8.3	[1.8-22.5]													2.8	55.6	33.3				8.3	
	Pork	Danish	0	[0-4.3]													11.9	73.8	14.3					
		Imported	4.4	[1.2-10.9]													19.8	71.4	4.4				4.4	
Gentamicin	Broiler meat	Danish	0	[0-6.1]											89.8	10.2								
		Imported	1.0	[0.02-5.2]											96.2	2.9						1.0		
	Beef	Danish	0	[0-12.8]											96.3	3.7								
		Imported	0	[0-9.7]											91.7	8.3								
	Pork	Danish	1.2	[0.03-6.5]											96.4	2.4						1.2		
		Imported	2.2	[0.3-7.7]											97.8							2.2		
Kanamycin	Broiler meat	Danish	0	[0-6.1]														100						
		Imported	18.3	[11.4-27.1]														81.7					18.3	
	Beef	Danish	3.7	[0.09-19.0]														96.3					3.7	
		Imported	2.8	[0.07-14.5]														97.2					2.8	
	Pork	Danish	2.4	[0.3-8.3]														96.4	1.2				2.4	
		Imported	3.3	[0.7-9.3]														96.7					3.3	
Ciprofloxacin	Broiler meat	Danish	0	[0-6.1]						27.1	72.9													
		Imported	1.0	[0.02-5.2]						21.2	73.1	4.8				1.0								
	Beef	Danish	0	[0-12.8]						22.2	66.7	11.1												
		Imported	0	[0-9.7]						30.6	69.4													
	Pork	Danish	0	[0-4.3]						21.4	67.9	10.7												
		Imported	1.1	[0.03-6.0]						31.9	64.8	2.2					1.1							
Vancomycin	Broiler meat	Danish	0	[0-6.1]							49.2	37.3	13.6											
		Imported	0	[0-3.5]							28.8	66.3	4.8											
	Beef	Danish	0	[0-12.8]							74.1	22.2	3.7											
		Imported	0	[0-9.7]							41.7	52.8	5.6											
	Pork	Danish	0	[0-4.3]							42.9	48.8	8.3											
		Imported	0	[0-4.0]							53.8	44.0	2.2											
Teicoplanin	Broiler meat	Danish	0	[0-6.1]					100															
		Imported	0	[0-3.5]					100															
	Beef	Danish	0	[0-12.8]					100															
		Imported	0	[0-9.7]					97.2	2.8														
	Pork	Danish	0	[0-4.3]					100															
		Imported	0	[0-4.0]					97.8	2.2														
Linezolid	Broiler meat	Danish	0	[0-6.1]							16.9	83.1												
		Imported	0	[0-3.5]						1.9	31.7	66.3												
	Beef	Danish	0	[0-12.8]							14.8	85.2												
		Imported	0	[0-9.7]							13.9	86.1												
	Pork	Danish	0	[0-4.3]							28.6	69.0	2.4											
		Imported	0	[0-4.0]							19.8	80.2												
Salinomycin	Broiler meat	Danish	1.7	[0.04-9.1]								89.8	8.5	1.7										
		Imported	0	[0-3.5]								94.2	5.8											
	Beef	Danish	0	[0-12.8]								100												
		Imported	0	[0-9.7]								100												
	Pork	Danish	0	[0-4.3]								100												
		Imported	0	[0-4.0]								100												

Vertical solid lines indicate EUCAST epidemiological cut-off values except for ciprofloxacin, kanamycin and salinomycin where the cut-off values were set by DANMAP. EUCAST/CLSI clinical breakpoints indicated as vertical dotted lines if different from the corresponding epidemiological cut-off values. See table AP2.2 for further details

White fields represent the range of dilutions tested. MIC values equal to or lower than the lowest concentration tested are presented as the lowest concentration. MIC values greater than the highest concentration in the range are presented as one dilution step above the range

Table AP1.22. Distribution of MICs and resistance (%) in *Escherichia coli* from broilers (n=118), cattle (n=106) and pigs (n=160), Denmark

DANMAP 2010

Antimicrobial agent	Animal species	% Resistant	95% Confidence interval	Distribution (%) of MICs																	
				0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	>2048
Tetracycline	Broilers	15.3	[9.3-23.0]								83.9	0.8				15.3						
	Cattle	9.4	[4.6-16.7]								74.5	16.0			0.9	8.5						
	Pigs	36.9	[29.4-44.9]								58.1	4.4	0.6	1.3	0.6	35.0						
Chloramphenicol	Broilers	2.5	[0.5-7.3]								4.2	48.3	44.9		0.8	1.7						
	Cattle	0.9	[0.02-5.1]									34.9	64.2				0.9					
	Pigs	4.4	[1.8-8.8]								1.9	43.8	49.4	0.6	3.1		1.3					
Florfenicol	Broilers	0.8	[0.02-4.6]								5.1	43.2	50.0	0.8			0.8					
	Cattle	0.9	[0.02-5.1]									33.0	65.1	0.9			0.9					
	Pigs	0	[0-2.3]								1.3	37.5	58.1	3.1								
Ampicillin	Broilers	21.2	[14.2-29.7]							4.2	28.8	43.2	2.5		0.8	20.3						
	Cattle	3.8	[1.0-9.4]							6.6	28.3	55.7	5.7			3.8						
	Pigs	23.1	[16.8-30.4]							3.8	28.1	42.5	2.5			23.1						
Ceftiofur	Broilers	0	[0-3.1]						94.1	5.9												
	Cattle	0	[0-3.4]						99.1	0.9												
	Pigs	1.2	[0.2-4.4]						98.8		0.6			0.6								
Cefotaxime	Broilers	0	[0-3.1]				95.8	4.2														
	Cattle	0	[0-3.4]				100															
	Pigs	1.2	[0.2-4.4]				97.5	1.3			0.6		0.6									
Trimethoprim	Broilers	7.6	[3.5-14.0]							92.4						7.6						
	Cattle	0.9	[0.02-5.1]							99.1						0.9						
	Pigs	21.2	[15.2-28.4]							78.1	0.6					21.3						
Sulfonamide	Broilers	20.3	[13.5-28.7]													78.8	0.8				20.3	
	Cattle	4.7	[1.5-10.7]													95.3					4.7	
	Pigs	31.9	[24.7-39.7]													68.1					31.9	
Streptomycin	Broilers	14.4	[8.6-22.1]										61.0	24.6	2.5	2.5	3.4	5.9				
	Cattle	5.7	[2.1-11.9]										82.1	12.3		1.9	2.8	0.9				
	Pigs	46.9	[39.0-54.9]										45.0	8.1	5.0	7.5	10.6	23.8				
Gentamicin	Broilers	0	[0-3.1]						11.9	75.4	12.7											
	Cattle	0	[0-3.4]						51.9	45.3	2.8											
	Pigs	0.6	[0.02-3.4]						30.0	64.4	5.0		0.6									

Table AP1.22 (Continued). Distribution of MICs and resistance (%) in *Escherichia coli* from broilers (n=118), cattle (n=106) and pigs (n=160), Denmark

DANMAP 2010

Antimicrobial agent	Animal species	% Resistant	95% Confidence interval	Distribution (%) of MICs																	
				0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	>2048
Neomycin	Broilers	0.8	[0.02-4.6]								71.2	28.0				0.8						
	Cattle	0.9	[0.02-5.1]								97.2	1.9				0.9						
	Pigs	7.5	[3.9-12.7]								82.5	10.0				7.5						
Apramycin	Broilers	0.8	[0.02-4.6]									29.7	66.1	3.4	0.8							
	Cattle	0	[0-3.4]									63.2	35.8	0.9								
	Pigs	0.6	[0.02-3.4]									52.5	43.8	3.1		0.6						
Ciprofloxacin	Broilers	8.5	[4.1-15.0]	44.9	46.6		1.7	5.9	0.8													
	Cattle	0	[0-3.4]	63.2	36.8																	
	Pigs	0	[0-2.3]	71.3	28.8																	
Nalidixic acid	Broilers	8.5	[4.1-15.0]									91.5			1.7	3.4	3.4					
	Cattle	0	[0-3.4]									100										
	Pigs	0	[0-2.3]									100										
Colistin	Broilers	0	[0-3.1]							100												
	Cattle	0	[0-3.4]							100												
	Pigs	0.6	[0.02-3.4]							98.8	0.6		0.6									
Spectinomycin	Broilers	5.1	[1.9-10.7]											68.6	24.6	1.7		1.7	3.4			
	Cattle	1.9	[0.2-6.6]											84.9	9.4	3.8	0.9	0.9				
	Pigs	25.0	[18.5-32.4]											48.1	16.3	10.6	3.1	13.8	8.1			

Vertical solid lines indicate EUCAST epidemiological cut-off values except for apramycin and sulfonamide where the cut-off values were set by DANMAP. EUCAST/CLSI clinical breakpoints indicated as vertical dotted lines if different from the corresponding epidemiological cut-off values. See table AP2.2 for further details

White fields represent the range of dilutions tested. MIC values equal to or lower than the lowest concentration tested are presented as the lowest concentration. MIC values greater than the highest concentration in the range are presented as one dilution step above the range

Table AP1.23. Distribution of MICs and resistance (%) in *Escherichia coli* from broiler meat (Danish n=158; imported n=177), beef (Danish n=32; imported n=39) and pork (Danish n=68; imported n=50), Denmark

DANMAP 2010

Antimicrobial agent	Food type	Origin	% Resistant	95% Confidence interval	Distribution (%) of MICs																	
					0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	>2048
Tetracycline	Broiler meat	Danish	13.3	[8.4-19.6]								86.1	0.6			0.6	12.7					
		Imported	45.8	[38.3-53.4]								52.0	2.3		0.6		45.2					
	Beef	Danish	3.1	[0.08-16.2]								90.6	6.3				3.1					
		Imported	10.3	[2.9-24.2]								87.2	2.6				10.3					
	Pork	Danish	23.5	[14.1-35.4]								75.0	1.5			1.5	22.1					
		Imported	56.0	[41.3-70.0]								40.0	2.0	2.0			56.0					
Chloramphenicol	Broiler meat	Danish	0.6	[0.02-3.5]								2.5	53.8	42.4	0.6	0.6						
		Imported	20.9	[15.2-27.6]									37.9	40.1	1.1	12.4	1.1	7.3				
	Beef	Danish	0	[0-10.9]								6.3	28.1	65.6								
		Imported	0	[0-9.0]									35.9	61.5	2.6							
	Pork	Danish	2.9	[0.4-10.2]								7.4	35.3	50.0	4.4	2.9						
		Imported	8.0	[2.2-19.2]								4.0	50.0	38.0				8.0				
Florfenicol	Broiler meat	Danish	0	[0-2.3]								2.5	52.5	43.7	1.3							
		Imported	0.6	[0.01-3.1]								1.1	36.2	54.2	7.9			0.6				
	Beef	Danish	0	[0-10.9]								6.3	21.9	71.9								
		Imported	0	[0-9.0]									38.5	61.5								
	Pork	Danish	0	[0-5.3]								8.8	27.9	61.8	1.5							
		Imported	2.0	[0.05-10.6]								4.0	44.0	50.0				2.0				
Ampicillin	Broiler meat	Danish	16.5	[11.0-23.2]							8.9	42.4	31.6	0.6			16.5					
		Imported	58.2	[50.6-65.5]							1.1	21.5	17.5	1.7			58.2					
	Beef	Danish	3.1	[0.08-16.2]								15.6	71.9	9.4			3.1					
		Imported	5.1	[0.6-17.3]								35.9	56.4	2.6			5.1					
	Pork	Danish	23.5	[14.1-35.4]							5.9	32.4	30.9	7.4			23.5					
		Imported	36.0	[22.9-50.8]							4.0	32.0	26.0	2.0			36.0					
Ceftiofur	Broiler meat	Danish	0.6	[0.02-3.5]						99.4				0.6								
		Imported	6.8	[3.6-11.5]						92.7	0.6	0.6	2.8	0.6	2.8							
	Beef	Danish	0	[0-10.9]						100												
		Imported	2.6	[0.06-13.5]						94.9	2.6				2.6							
	Pork	Danish	1.5	[0.04-7.9]						97.1	1.5		1.5									
		Imported	0	[0-7.1]						100												
Cefotaxime	Broiler meat	Danish	0.6	[0.02-3.5]				99.4						0.6								
		Imported	6.8	[3.6-11.5]				91.5	1.7		1.1	1.7	1.1	2.8								
	Beef	Danish	0	[0-10.9]				100														
		Imported	2.6	[0.06-13.5]				97.4						2.6								
	Pork	Danish	1.5	[0.04-7.9]				97.1	1.5			1.5										
		Imported	0	[0-7.1]				100														
Trimethoprim	Broiler meat	Danish	4.4	[1.8-8.9]							95.6						4.4					
		Imported	40.7	[33.4-48.3]							59.3		1.1	0.6			39.0					
	Beef	Danish	3.1	[0.08-16.2]							96.9						3.1					
		Imported	2.6	[0.06-13.5]							97.4						2.6					
	Pork	Danish	16.2	[8.4-27.1]							83.8						16.2					
		Imported	30.0	[17.9-44.6]							70.0						30.0					
Sulfonamide	Broiler meat	Danish	15.2	[10.0-21.8]													84.8				0.6	14.6
		Imported	55.9	[48.3-63.4]													43.5	0.6			1.7	54.2
	Beef	Danish	6.2	[0.8-20.8]													93.8					6.3
		Imported	5.1	[0.6-17.3]													94.9				2.6	2.6
	Pork	Danish	19.1	[10.6-30.5]													80.9				1.5	17.6
		Imported	36.0	[22.9-50.8]													64.0				2.0	34.0

Streptomycin	Broiler meat	Danish	15.2	[10.0-21.8]											67.7	17.1		5.1	3.2	7.0			
		Imported	45.8	[38.3-53.4]											39.5	14.7	5.1	7.9	7.9	24.9			
	Beef	Danish	3.1	[0.08-16.2]											81.3	15.6			3.1				
		Imported	5.1	[0.6-17.3]											92.3	2.6			2.6	2.6			
	Pork	Danish	38.2	[26.7-50.8]											52.9	8.8	4.4	8.8	16.2	8.8			
		Imported	56.0	[41.3-70.0]											36.0	8.0	2.0	4.0	18.0	32.0			
Gentamicin	Broiler meat	Danish	0	[0-2.3]						24.7	66.5	8.9											
		Imported	2.8	[0.9-6.5]						23.2	66.1	7.9					2.8						
	Beef	Danish	0	[0-10.9]						25.0	71.9	3.1											
		Imported	2.6	[0.06-13.5]						41.0	51.3	5.1					2.6						
	Pork	Danish	1.5	[0.04-7.9]						32.4	58.8	7.4			1.5								
		Imported	0	[0-7.1]						26.0	62.0	12.0											
Neomycin	Broiler meat	Danish	0.6	[0.02-3.5]								79.7	19.6					0.6					
		Imported	10.2	[6.1-15.6]								77.4	11.3	1.1	0.6			9.6					
	Beef	Danish	0	[0-10.9]								96.9	3.1										
		Imported	0	[0-9.0]								92.3	5.1	2.6									
	Pork	Danish	2.9	[0.4-10.2]								80.9	11.8	4.4				2.9					
		Imported	4.0	[0.5-13.7]								76.0	18.0	2.0				4.0					
Apramycin	Broiler meat	Danish	0	[0-2.3]									42.4	53.8	3.8								
		Imported	0	[0-2.1]									42.4	54.8	2.8								
	Beef	Danish	0	[0-10.9]									46.9	53.1									
		Imported	0	[0-9.0]									59.0	41.0									
	Pork	Danish	1.5	[0.04-7.9]									50.0	42.6	5.9			1.5					
		Imported	0	[0-7.1]									42.0	52.0	6.0								
Ciprofloxacin	Broiler meat	Danish	3.8	[1.4-8.1]	66.5	29.7		1.3	2.5						4.0								
		Imported	40.7	[33.4-48.3]	33.3	26.0		2.8	18.6	10.7	2.3	1.7	0.6										
	Beef	Danish	0	[0-10.9]	62.5	37.5																	
		Imported	5.1	[0.6-17.3]	71.8	23.1			2.6						2.6								
	Pork	Danish	1.5	[0.04-7.9]	63.2	35.3	1.5																
		Imported	4.0	[0.5-13.7]	64.0	32.0			2.0						2.0								
Nalidixic acid	Broiler meat	Danish	3.8	[1.4-8.1]									96.2					1.3	2.5				
		Imported	37.9	[30.7-45.4]									59.3	1.7	1.1			3.4	34.5				
	Beef	Danish	0	[0-10.9]									100										
		Imported	5.1	[0.6-17.3]									89.7	5.1					5.1				
	Pork	Danish	1.5	[0.04-7.9]									98.5					1.5					
		Imported	4.0	[0.5-13.7]									96.0						4.0				
Colistin	Broiler meat	Danish	0	[0-2.3]							100												
		Imported	6.2	[3.1-10.8]							93.8		2.8	3.4									
	Beef	Danish	0	[0-10.9]							100												
		Imported	0	[0-9.0]							97.4	2.6											
	Pork	Danish	0	[0-5.3]							100												
		Imported	0	[0-7.1]							100												
Spectinomycin	Broiler meat	Danish	3.8	[1.4-8.1]												80.4	11.4	4.4	0.6	1.9	1.3		
		Imported	29.4	[22.8-36.7]												46.9	16.4	7.3	1.7	12.4	15.3		
	Beef	Danish	0	[0-10.9]												78.1	15.6	6.3					
		Imported	2.6	[0.06-13.5]												84.6	7.7	5.1	2.6				
	Pork	Danish	19.1	[10.6-30.5]												60.3	17.6	2.9	1.5	11.8	5.9		
		Imported	14.0	[5.8-26.7]												52.0	22.0	12.0		10.0	4.0		

Vertical solid lines indicate EUCAST epidemiological cut-off values except for apramycin and sulfonamide where the cut-off values were set by DANMAP. EUCAST/CLSI clinical breakpoints indicated as vertical dotted lines if different from the corresponding epidemiological cut-off values. See table AP2.2 for further details

White fields represent the range of dilutions tested. MIC values equal to or lower than the lowest concentration tested are presented as the lowest concentration. MIC values greater than the highest concentration in the range are presented as one dilution step above the range

Table AP1.24. Distribution of MICs and resistance (%) in *Escherichia coli* from diagnostic pigs (n=33), Denmark

DANMAP 2010

Antimicrobial agent	Animal species	% Resistant	95% Confidence interval	Distribution (%) of MICs																	
				0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	>2048
Tetracycline	Pigs	69.7	[51.3-84.4]								27.3	3.0			6.1	63.6						
Chloramphenicol	Pigs	9.1	[1.9-24.3]									72.7	18.2		3.0		6.1					
Florfenicol	Pigs	3.0	[0.08-15.8]								9.1	66.7	18.2	3.0			3.0					
Ampicillin	Pigs	42.4	[25.5-60.8]							12.1	33.3	12.1				42.4						
Ceftiofur	Pigs	3.0	[0.08-15.8]						97.0					3.0								
Cefotaxime	Pigs	3.0	[0.08-15.8]				97.0						3.0									
Trimethoprim	Pigs	51.5	[33.5-69.2]							45.5	3.0					51.5						
Sulfonamide	Pigs	78.8	[61.1-91.0]													21.2					78.8	
Streptomycin	Pigs	75.8	[57.7-88.9]										21.2	3.0	3.0	21.2	18.2	33.3				
Gentamicin	Pigs	3.0	[0.08-15.8]						78.8	15.2	3.0				3.0							
Neomycin	Pigs	18.2	[7.0-35.5]								72.7	9.1				18.2						
Apramycin	Pigs	3.0	[0.08-15.8]									90.9	6.1			3.0						
Ciprofloxacin	Pigs	24.2	[11.1-42.3]	66.7	9.1	3.0		12.1	9.1													
Nalidixic acid	Pigs	21.2	[9.0-38.9]									78.8					21.2					
Colistin	Pigs	0	[0-10.6]							100												
Spectinomycin	Pigs	63.6	[45.1-79.6]											21.2	6.1	9.1	6.1	12.1	45.5			

Vertical solid lines indicate EUCAST epidemiological cut-off values except for apramycin and sulfonamide where the cut-off values were set by DANMAP. EUCAST/CLSI clinical breakpoints indicated as vertical dotted lines if different from the corresponding epidemiological cut-off values. See table AP2.2 for further details

White fields represent the range of dilutions tested. MIC values equal to or lower than the lowest concentration tested are presented as the lowest concentration. MIC values greater than the highest concentration in the range are presented as one dilution step above the range

APPENDIX 2

List of abbreviations

ACD	Defined Animal Course Dose
ADD	Defined Animal Daily Dose
ADD_{kg}	Defined Animal Daily Dose per kg animal
AGP	Antimicrobial Growth Promoter
ATC	Anatomical Therapeutic Chemical Classification System
CHR	Central Husbandry Register
CI	Confidence Interval
CNS	Central Nervous System
CPR	Danish Civil Registry
DAD	Defined Daily Doses per 100 admissions
DBD	Defined Daily Doses per 100 occupied bed-days
DCM	Department of Clinical Microbiology
DID	Defined Daily Doses per 1,000 inhabitants per day
DDD	Defined Daily Dose
DMA	Danish Medicines Agency
DTU	Technical University of Denmark
DVFA	Danish Veterinary and Food Administration
EARS-Net	The European Antimicrobial Resistance Surveillance Network
ESBL	Extended Spectrum Beta-Lactamase
GI	Gastrointestinal
GP	General Practitioner
HLGR	High-level gentamicin resistance
MIC	Minimum Inhibitory Concentration
MRSA	Methicillin-resistant *Staphylococcus aureus*
N	Number of samples
n	Number of isolates tested for antimicrobial susceptibility
OIE	World Organisation for Animal Health
PMWS	Postweaning multisystemic wasting syndrome
RFCA	Regional Veterinary and Food Control Authorities
SSI	Statens Serum Institut
VetStat	Danish Register of Veterinary Medicines
VRE	Vancomycin Resistant Enterococci
WHO	World Health Organization
WT	Wild type

List of words

Anatomical Therapeutic Chemical (ATC) classification. International classification system for drug consumption studies. The ATC code identifies the therapeutic ingredient(s) of each drug for human use according to the organ or system on which it acts and its chemical, pharmacological and therapeutic properties. Antibacterials for systemic use are known as ATC group J01. The ATC classification is maintained by the WHO Collaborating Centre for Drug Statistics and Methodology (Oslo, Norway) (http://www.whocc.no/atcddd/indexdatabase/). The ATC classification for veterinary medicinal products, ATCvet, is based on the same main principles as the ATC classification system for medicines for human use and is also maintained by the WHO Collaborating Centre for Drug Statistics and Methodology (http://www.whocc.no/atcvet/database/).

Antibacterial agents. Synthetic (chemotherapeutics) or natural (antibiotics) substances that destroy bacteria or suppress bacterial growth or reproduction [Source: Dorland's Illustrated Medical Dictionary]. Antimycobacterial agents are not included. Only antibacterial agents for systemic use are included (J01 in the ATC system) in the section on human consumption.

Antimicrobial agents. The term 'antimicrobial agents' covers antibacterial, antiviral, coccidiostatic and antimycotic agents. In the section on veterinary consumption, the broad term 'antimicrobial agents' is usually used because coccidiostats are included. Antiviral substances are not used in veterinary medicine, and antimycotics are only registered for topical veterinary use and used mainly in companion animals. Antimycobacterial agents are not included. The term 'antibacterial agents' is only used in the veterinary section for precision, to distinguish from use of coccidiostats as feed additives (poultry only).

Broiler. A type of chicken raised specifically for meat production. In Denmark, the average weight after slaughter is 1.66 kg.

Central Husbandry Register (CHR). This is a register of all Danish farms defined as geographical sites housing production animals. It contains numerous information concerning ownership, farm size, animal species, age groups, number of animals and production type. Each farm has a unique farm identity number (CHR-number).

Defined Daily Dose (DDD). This is the assumed average maintenance dose per day in adults. It should be emphasised that the Defined Daily Dose is a unit of measurement and does not necessarily reflect the recommended or prescribed daily dose. DDDs provide a fixed unit of measurement independent of price and formulation, enabling the assessment of trends in drug consumption and to perform comparisons between population groups. The DDDs are defined and revised yearly by the WHO Collaborating Centre for Drug Statistics and Methodology (http://www.whocc.no/atcddd/indexdatabase/). DDD/1,000 inhabitant-days is called DID.

Defined Animal Daily Dose (ADD and ADDkg). This is a national veterinary equivalent to the DDD. This is an assumed average daily dose per animal, defined as the daily maintenance dose for a drug used for its main indication in a specified species. The dose is defined for a "standard animal", i.e. an animal with an estimated average weight within a specified age group. In VetStat, ADDs are calculated for each age group. Otherwise, the general principles for standardisation of dosage for animals are similar to that used by the WHO Collaborating Centre for Drug Statistics and Methodology to calculate Defined Daily Dose (DDD) in humans [Jensen *et al.* 2004. Prev Vet Med. 64: 201-215]. The ADD_{kg} is the ADD per kg animal. Consumption calculated in ADD_{kg} allows summation of consumption across different age groups and animal species.

Defined Animal Course Dose (ACD and ACD_{kg}). The duration of the treatment related to one application may vary substantially between antimicrobial drugs. To correct for this, total course dose has been introduced as unit of measurement for antimicrobial usage. As a standard, the length of the course is here defined as 6 days, if nothing else is stated. Course doses are assigned per kilogram (live weight) of the animal species (ACD_{kg}) or age group of the relevant species (ADC_{xx}) and are based on the corresponding ADD_{kg} or ADD_{xx}, respectively, for the relevant animal species and drug formulations.

ESBL. In this DANMAP report 'ESBL' describes the clinically important acquired beta-lactamases with activity against extended-spectrum cephalosporins; including the classical class A ESBLs (CTX-M, SHV, TEM), the plasmid-mediated AmpC and OXA-ESBLs [Giske *et al*. 2009. J Antimicrob Chemother. 63: 1-4].

Finishers. Pigs from 30 kilogram live weight to time of slaughter at app. 100 kilogram live weight.

Fully sensitive. See definition of multi-resistance.

Heifer. A young female cow before first calving.

Intramammaria. Antimicrobial agents for local application in the mammary gland (udder) to treat mastitis.

Intramammary syringe. A one dose applicator for use in the udder.

Layer. A hen raised to produce eggs for consumption.

Minimum Inhibitory Concentration (MIC). This is the lowest concentration of antimicrobial agent in a given culture medium, e.g. broth or agar, below which growth of the bacteria is not inhibited.

Multi-resistant. A *Salmonella* or *E. coli* isolate is assumed multi-resistant if resistant to three or more of the following ten antimicrobial groups: Tetracyclines (tetracycline), phenicoles (chloramphenicol and/or florfenicol), penicillins (ampicillin), cephalosporins (ceftiofur and/or cefotaxime), sulfonamides (sulfonamide), trimethoprim (trimethoprim), aminoglycosides - except streptomycin (gentamicin), streptomycin (streptomycin), quinolones (ciprofloxacin and/or nalidixic acid) and polymycins (colistin). An isolate will be referred to as fully sensitive if susceptible to all the above listed antimicrobial groups.

Pet animals. Dogs, cats, birds, mice, guinea pigs and more exotic species kept at home for pleasure, rather than one kept for work or food; does not include horses.

Piglet. The newborn pig is called at piglet from birth till they are permanently separated from the sow at 3-4 weeks of age. The weight of the piglet at weaning is approximately 7 kilogram.

Poultry. The major production species are fowl - *Gallus gallus* (broilers, layers, including breeding and rearing) and turkey. Regarding antimicrobial consumption, 'poultry' also includes domesticated ducks, geese, game birds and pigeons. In DANMAP 2010, the poultry isolates originated from *Gallus gallus* and broiler meat, and a minor part originated from imported turkey meat.

Significant. When written in the text, significant differences imply statistically significant differences where $p<0.05$ using Chi-square or Fisher's Exact Test when the number of samples is low (<25).

Sow. Any breeding female pig that has been served and is on the farm.

Steer. Castrated male cattle, usually young animal.

Weaner. Any pig, 7–30 kilogram live weight.

Wild type. The typical form of an organism, strain, gene, or characteristic as it occurs in nature.

Materials and methods

1. General information

For the 2010 DANMAP report, the population and geographical data were obtained from Statistics Denmark (www.dst.dk) and the data on general practitioners from the Danish Medical Association (www.laeger.dk).

In this report, the epidemiological unit for pigs, cattle and broilers was defined as the individual farm, meaning that only one isolate per bacterial species per farm was included in the report. For humans, the epidemiological unit was defined as the individual patient and the first isolate per patient per year was included. For food, the epidemiological unit was defined as the individual meat sample.

2. Data on antimicrobial consumption

Antimicrobial agents used for humans and animals in Denmark are presented in Table 3.2.

2.1. Antimicrobial consumption in animals

Since 2001, consumption data presented in this report were obtained from the national monitoring program, VetStat. Prior to 2001, data were based on overall sales figures from the pharmaceutical industry (Table AP1.0).

In Denmark, all therapeutic drugs are prescription-only and VetStat collects data on all medicines prescribed by veterinarians for use in animals. In addition, data on consumption of coccidiostatics as feed additives (non-prescription) and antimicrobial growth promoters (no longer in use) are collected by VetStat. Data on coccidiostatics were reported until 2004, but due to problems in data transfer data were not reported in 2005 and 2006. Data on coccidiostatics for 2007–2010 will be presented in later reports following validation of data.

Until 2007, antimicrobial agents could only be purchased at the pharmacy or in medicated feed from the feed mills. In 2010, sales from feed mills comprised almost entirely prescriptions for aquaculture and sales of zinc chloride for the pig production. The pharmacy either sells the medicines to veterinarians for own use in practice or for re-sale to farmers, or sells the medicines directly to the animal holder on presentation of a prescription. By law, the profit that veterinarians may make on the sale of medicine is very small (5%), thereby limiting the economic incentive to sell medicine. Hence, in 2010, only 10% of the antimicrobial agents used for animals were used or distributed by veterinarians.

From April 2007, the monopoly was suspended and private companies (two in 2010) can now, on certain conditions (identical to the pharmacies), sell prescribed veterinary medical products for production animals. In addition, price setting was liberalised, which allowed for discounts corresponding to lower administration cost related to sale of large quantities to the veterinarians. In 2010, the animal owners and veterinarians purchased the antimicrobial agents equally from the pharmacies (49%) and the veterinary drug trading companies (49%), while only 2.4% was purchased from the feed mills.

Data on all sales of veterinary prescription medicine from the pharmacies, private companies, feed mills and veterinarians are sent electronically to VetStat. Veterinarians are required by law to report to VetStat the use of all prescription medicines in production animals on a monthly basis. For most veterinarians, the registration of data is linked to the writing of invoices. For the DANMAP report the amount of drugs reported by the veterinary practitioners is validated against pharmacy data on the total sales of therapeutic drugs for use in practice. The electronic registration of the sales at the pharmacies is linked to the billing process, which ensures a high data quality regarding amounts and identity of drugs.

The VetStat database contains detailed information about source and consumption for each prescription item: date of sale, identity of prescribing veterinarian, source ID (identity of the pharmacy, feed mill, or veterinarian reporting), package identity code and amount, animal species, age-group, disease category and code for farm-identity (CHR - Danish Central Husbandry Register). The package code is a unique identifier, relating to all information on the medicine, such as active ingredient, content as number of unit doses (e.g. number of tablets), package size, and code of the antimicrobial agent in the Veterinary Anatomical Therapeutic Chemical (ATCvet) classification system.

Knowledge of the target animal species enables the presentation of consumption data in Defined Animal Daily Doses (ADD). The ADD system is a national veterinary equivalent to the international Defined Daily Doses (DDD) system applied in the human field [www.whocc.no]. See further about the ADD system in the DANMAP 2009 report.

The consumption is compared with production in kg meat or number of animals produced. Due to an increasing number of pigs exported around 30 kg, involving 25% of pigs produced in 2010, an adjusted measure of consumption per pig was calculated. The adjustment is based on the assumption that pigs exported at 30 kg, on average received the same amount of antimicrobial agents before export, as other pigs from farrowing to 30 kg:

Antimicrobial use per pig produced (adjusted)= $(ADD_s * (N_f/N_w) + ADD_w * (N_f/N_w) + ADD_f) / N_f$,

where ADD_s = Amounts of antimicrobial used in sow herds, measured in ADD_{kg} ; ADD_w = Amounts of antimicrobial used in weaning pigs herds, measured in ADD_{kg} ; ADD_f = Amounts of antimicrobial used in finisher pigs, measured in ADD_{kg} ; N_w = Number of pigs produced to 30 kg bodyweight, including pigs exported at 15-50 kg (mostly at 30 kg); N_f = Number of pigs produced to slaughter, whether exported domestically or exported.

2.2. Antimicrobial consumption in humans

Consumption data presented in this report were obtained from the Danish Medicines Agency (DMA) (http://www.laegemiddelstyrelsen.dk). The DMA has the legal responsibility for monitoring the consumption of all human medicinal products. This is carried out by monthly reporting of consumption from all pharmacies in Denmark, including hospital pharmacies, to the DMA. Data from the primary health care sector have been collected since 1994, whereas data on consumption in hospitals are available from 1997.

Certain categories of hospitals were excluded when the consumption was measured by occupied bed-days and admissions. This year, data from private hospitals and clinics, psychiatric hospitals, specialised non-acute care clinics, rehabilitation centres and hospices were excluded from DANMAP (representing approximately 3% of the antimicrobial consumption at hospitals and of the number of bed-days).

In Denmark, all antimicrobial agents for human use are prescription-only medicines and are sold by pharmacies in defined packages. Each package is uniquely identified by a code which can be related to package size, content as number of unit doses (e.g. number of tablets), content as number of Defined Daily Doses (DDD), code of the antimicrobial agent in the Anatomical Therapeutic Chemical (ATC) classification system, and producer. In addition, the following information is collected for each transaction: social security number (CPR number) of the patient, code identifying the prescribing physician, date and place (pharmacy, hospital pharmacy, institution) of the transaction, and information regarding reimbursement of cost, if applicable. Information on the indication for the prescription is not yet available. The data are transferred monthly to the DMA in an electronic format.

The present report includes data on the consumption of antibacterial agents for systemic use, or group J01, of the 2010 update of the ATC classification, in primary health care and in hospitals. As recommended by the World Health Organization (WHO), consumption of antibacterial agents in primary health care is expressed as a number of DDDs per 1,000 inhabitants and per day (DDD/1,000 inhabitant-days). Consumption in primary health care is also reported as a number of packages per 1,000 inhabitants. Consumption of antibacterial agents in hospitals is expressed as a number of DDDs per 1,000 inhabitants and per day (DDD/1000 inhabitant-days) to compare with primary health care and as a number of DDDs per 100 occupied bed-days and per day (DDD/100 occupied bed-days). Since antimicrobial consumption expressed as DDD/100 occupied bed-days does not necessarily reflect changes in hospital activity and production, consumption in hospitals is also presented as DDD/100 admitted patients.

The number of occupied bed-days is calculated as the date of discharge minus the date of admission (minimum one day), and the number of admissions is calculated as one admission whenever a patient is admitted to one specific ward (one patient can be registered as admitted multiple times if transferred between wards during one hospital stay). Data on the number of occupied bed-days (or patient-days) and number of admissions in each hospital were obtained from the National Patient Registry at the National Board of Health [http://www.sst.dk].

3. Collection of bacterial isolates

3.1. Animals

Animal isolates included in DANMAP 2010 from healthy production animals at slaughter, were *Escherichia coli, Enterococcus faecium, Enterococcus faecalis, Campylobacter coli* and *Campylobacter jejuni.* Isolates of *E. coli* O149 and *E. coli* F5 (K99) were collected from diagnostic submissions and *Salmonella* isolates were collected from subclinical infections as well as from cases of clinical salmonellosis.

***Campylobacter,* indicator *E. coli* and enterococci.** Samples from healthy pigs, cattle and broilers were collected at slaughter for the DANMAP programme by meat inspection staff or company personnel and sent for examination to the National Food Institute. For broilers, cloacal swab samples were collected weekly throughout the year representing all broiler flocks in Denmark (approximately 400 samples per year). In Denmark, a farm consists typically of more than 1 flock (2–12 flocks), and even though most of the flock samples were analysed only one isolate per farm of each bacterial species was finally included in the DANMAP report.

For pigs and cattle, the slaughter plants included in the DANMAP programme accounted for 94% and 90%, respectively, of the total number of animals slaughtered in Denmark during 2010. The number of pig and cattle samples taken at the slaughter plant was proportional to the number of animals slaughtered at each plant per year and samples were collected once a month from January through November as ceacum samples from pigs and rectum samples from cattle. As for broilers, only one isolate per farm of each bacterial species was included in the DANMAP report. Accordingly, the bacterial isolates may be regarded as representing a stratified random sample of the respective populations, and the observed prevalence of resistant isolates provides an estimate of the true occurrence in the population.

An overview of the number of samples analysed, the

number of isolates obtained and the number of MIC-determinations for pigs, cattle and broilers is presented in Table AP2.1. For *Campylobacter,* the isolation rate of *C. jejuni* from pigs and of *C. coli* from cattle and broilers was low and MIC-determinations were therefore not performed. Samples from cattle were not analysed for enterococci.

Isolates from diagnostic submissions were collected for the DANMAP programme at both the National Food Institute and at the Laboratory of Swine Diseases, the Danish Agriculture & Food Council, Kjellerup. *E. coli* O149 from diarrhoeic pigs and *E. coli* from diarrhoeic cattle were included, with no more than one isolate representing each farm. However, *E. coli* F5(K99) was not reported in 2010 due to the low number of isolates available. *Staphylococcus hyicus* isolates from skin infections in pigs were also collected, but not reported in 2009 and 2010 due to the low number of isolates. Data on *S. hyicus* collected over three years are expected to be reported in the next DANMAP report.

***Salmonella*.** The National Food Institute is the national reference laboratory for *Salmonella* in animals, feeding stuffs and food, and therefore receives all isolates for typing. Among all serotyped *Salmonella* isolates, one isolate per farm was selected for the DANMAP report. Only isolates of *S.* Typhimurium and *S.* Enteritidis were included in DANMAP. In general, isolates of *S.* Typhimurium include the monophasic variants with antigenic formulas S. 4,5,12:i:- and S. 4,12:i:-.

The majority of the *Salmonella* isolates from pigs (95% in 2010) originated from the Danish *Salmonella* surveillance programmes: The results of a serological surveillance at the slaughterhouses and in all breeding herds appointed risk herds to be further examined by analysing pen-faecal samples: 1) finisher herds at level 2 and level 3 farms (i.e. farms with high level of *S.* Typhimurium antibodies in three successive months in meat juice samples taken at slaughter), 2) related (supplying) sow herds, and finally 3) breeding and multiplier herds with high serum levels in three monthly samples. In 2010, 1,089 pig herds were appointed as risk herds from the sero-surveillance one or more times and *S.* Typhimurium was isolated from 434 of these herds (including the monophasic variants). In addition, *Salmonella* in samples from pig herds investigated due to clinical disease (not necessarily salmonellosis) were included (21 isolates in 2010).

For broilers, all flocks were sampled before slaughter as part of the *Salmonella* surveillance programme; this includes flocks intended for export. Samples were collected 15-21 days before slaughter. Since 2008, an additional AM (ante mortem)-testing of broiler flocks was introduced 7-10 days prior to slaughter. In 2010, 3,773 broiler flocks were analysed of which 43 were positive for *Salmonella*. Data are not presented in tables due to the low number of isolates in 2010.

For cattle, a total of 144 different herds were examined based on clinical indication. A total of 26 *Salmonella* were isolated, including seven *S.* Dublin and 18 *S.* Typhimurium isolates (including the monophasic variants *S.* 4,5,12:i:- and *S.* 4,12:i:- with six and one isolate, respectively).

Further details on the sampling procedures in the *Salmonella* surveillance programmes are described in the Annual Report on Zoonoses in Denmark, 2010.

3.2. Meat

***Campylobacter*, indicator *E. coli* and Enterococci.** The meat isolates originated from meat samples collected at wholesale and retail outlets by the Regional Veterinary and Food Control Authorities (RFCA) in all regions of Denmark. The samples were collected during the course of routine inspection carried out by the authorities or on specific request from the Danish Veterinary and Food Administration (DVFA) for the DANMAP programme. The collected material consisted of both Danish and imported meat. Indicator *E. coli* and enterococci were collected from beef, pork and poultry meat (118, 184 and 187 Danish samples; and 99, 175 and 226 imported samples, respectively). *Campylobacter* were only collected from poultry meat (72 Danish samples and 95 imported samples). The meat samples were collected according to the guidelines for microbiological examination of food from the DVFA [Vejledning nr. 9613 af 20. Dec. 2002 om offentlig mikrobiologisk kontrol af fødevarer]. Only one isolate per bacterial species per meat sample was selected for DANMAP.

Table AP2. 1. Number of DANMAP samples, isolates and MIC-tests from healthy production animals at slaughter, Denmark

DANMAP 2010

		E. coli	*E. faecium*	*E. faecalis*	*C. jejuni*	*C. coli*
Pigs	No. of samples analysed (1 per farm)	219	738	738	269	269
	No. of isolates	199	136	167	-	-
	No. of isolates MIC-tested/reported	160	133	157	0	103
Cattle	No. of samples analysed (1 per farm)	133	-	-	216	216
	No. of isolates	122	-	-	-	-
	No. of isolates MIC-tested/reported	106	-	-	98	0
Broilers	No. of samples analysed (no. of flocks)	382	382	382	382	382
	No. of farms represented	153	169	169	169	169
	No. of isolates MIC-tested/reported	118	119	112	41	0

Note: Data in this table should not be used for reportation of prevalences of the bacterial species

Salmonella. The *Salmonella* isolates from Danish pork and beef originated from the *Salmonella* surveillance programme, comprising swab samples of pork and beef carcasses taken at the slaughterhouses after cooling. In Danish pork, 22,485 pooled samples (each of five carcasses) were analysed in 2010, and an estimated 1.2% of the pig carcasses were *Salmonella* positive. In addition, 223 single animals were tested in smaller slaughterhouses, where 1.8% of the pig carcasses were *Salmonella* positive. In Danish beef, 7,660 pooled samples (each of five carcasses) were analysed in 2010, and an estimated 0.3% of the cattle carcasses were *Salmonella* positive. In addition, 162 single animals were tested in the smaller slaughterhouses, and here none of the cattle carcasses were *Salmonella* positive [Annual Report on Zoonoses in Denmark 2010]. All isolates of *S.* Typhimurium and *S.* Enteritidis from a positive batch of meat were included in this report.

Salmonella isolates from imported poultry meat and other imported fresh meats originated from a case-by-case risk assessment programme (Danish Veterinary and Food Administration). For each tested batch of meat, 12 pooled samples (1–60 single samples) were tested for *Salmonella*. All isolates of *S.* Typhimurium and *S.* Enteritidis within one batch of meat were included in this report. In 2010, 58 of 490 batches of imported broiler meat, 56 of 592 batches of imported turkey meat, 40 of 296 batches of imported pork, and four of 127 batches of imported beef were *Salmonella* positive. As the sampling is risk based, the findings are not indicative of the prevalence at retail [Annual Report on Zoonoses in Denmark 2010].

Isolates of *S.* Typhimurium include the monophasic variants with antigenic formula 4,5,12:i:- and 4,12:i:-.

3.3. Humans

***Salmonella enterica* serovars Typhimurium and Enteritidis and *Campylobacter jejuni*.** Antimicrobial susceptibility was performed on human faecal isolates submitted to Statens Serum Institut (SSI). *Campylobacter* isolates were submitted from Departments of Clinical Microbiology (DCM) covering three geographical regions: Northern Jutland, Funen and Roskilde/Køge. Information on travel history was obtained for these patients. *Salmonella* isolates were submitted from all DCM in Denmark. Exact figures of the proportion tested and the sampling strategy for the different species can be found in the corresponding chapters of this report.

Staphylococcus aureus. All blood isolates were referred to the *Staphylococcus* reference laboratory at SSI on a voluntary basis. In November 2006, methicillin resistant *S. aureus* (MRSA) became a notifiable disease in Denmark and since then it has been mandatory to send all MRSA isolates to the reference laboratory.

Invasive *Streptococcus pneumoniae, Streptococcus pyogenes* (group A streptococci), group B, C and G streptococci. Invasive pneumococcal disease is a notifiable disease in Denmark, and therefore all blood and spinal fluid isolates nationwide are sent to SSI for determination or confirmation as well as susceptibility testing and typing. Group A, B, C and G streptococcal isolates are referred to SSI on a voluntary basis.

E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*, invasive *Enterococcus faecium* and invasive *Enterococcus faecalis. Data were provided on all isolates recorded from either blood samples (*E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, E. faecium* and *E. faecalis*) or urine samples (*E. coli, Klebsiella pneumoniae*) submitted for susceptibility testing to the participating DCM at the following hospitals: Rigshospitalet, Hvidovre, Herlev, Hillerød, Slagelse, Næstved, Roskilde, Odense, Esbjerg, Vejle, Herning, Aarhus, Viborg, and Aalborg.

In 2010, no samples were collected from healthy humans.

4. Isolation and identification of bacteria

4.1. Animals

Salmonella. Examination of samples was done by non-selective pre-enrichment of 25 g material in a 1:10 dilution with buffered peptone water (BPW) and incubated 16-20 hours at 37°C. A plate with Modified Semi-solid Rappaport-Vassiliadis (MSRV) medium was inoculated with 0.1 ml of BPW deposited as 3 drops. After incubation overnight at 41.5°C, material from MSRV swarming zones were inoculated onto Brilliant Green Agar. Overnight incubation at 37°C was followed by serotyping of suspect colonies by slide agglutination. For cattle samples, in addition 1.0 ml of the BPW suspension was incubated in 9 ml selenite cystine broth overnight at 41.5°C before inoculation on MSRV agar.

Campylobacter. Samples from pigs and poultry were examined by direct inoculation on mCCD agar (Oxoid, Denmark) followed by incubation in micro-aerophilic atmosphere for 2-4 days at 41.5°C. For cattle, selective enrichment in Preston broth at a ratio of 1:10 incubated in microaerophilic atmosphere for 24 h at 41.5°C was performed followed by inoculation of 10 µl of the enrichment broth to mCCD agar. *Campylobacter* suspect colonies were verified by microscopy and oxidase activity (oxidase strips, Oxoid). Species-identification was performed by catalase activity and the ability to hydrolyse indoxyl acetate and hippurate. All isolates of *C. jejuni* and *C. coli* were stored (-80°C).

***E. coli* from healthy animals (indicator *E. coli*)**. The material was inoculated directly onto Drigalski agar (SSI Diagnostica, Denmark) and incubated at 37°C overnight. Yellow colonies were inoculated onto BBL CHROMagar Orientation Medium (Becton Dickinson, Germany) and red colonies were identified as *E. coli* after incubation at 37°C overnight.

Enterococci. One drop of material suspended in 2 ml sodium chloride (0.9%) was spread on Slanetz-Bartley agar and incubated for two days at 42°C. Up to four colonies with morphology typical of *E. faecalis* / *E. faecium* were sub-cultivated on blood agar. Colonies were identified by the following criteria: Colour, motility, arginine dihydrolase testing and the ability to ferment mannitol, sorbitol, arabinose, raffinose and melibiose. All isolates of *E. faecium* and *E. faecalis* were stored (-80°C). Like in previous years, no samples from cattle were investigated for enterococci.

Pathogens. The diagnostic submissions were examined according to the standard procedures at the participating laboratories.

4.2. Meat

Salmonella was isolated according to the guidelines for microbiological examination of food from the Danish Veterinary and Food Administration [NMKL No. 187, 2007]. Sero- and phage-typing was performed for all isolates at the National Food Institute.

Campylobacter was isolated according to the guidelines for microbiological examination of food from the DVFA [NMKL No. 119, 3rd ed., 2007]. Identification was performed at the Regional Veterinary and Food Control Authorities (RFCA) by microscopy or test kit DRO150M (Oxoid), and oxidase activity (except for one of the laboratories), catalase activity, and the ability to hydrolyse indoxyl acetate and hippurate. All isolates of *C. jejuni, C. coli* and *C. lari* were stored (-80°C) and sent to the National Food Institute for MIC-testing of *C. jejuni* and *C. coli.*

Indicator *E. coli* was isolated by adding 5 g of the sample to 45 ml of MacConkey- or laurylsulfphate-broth, which was incubated overnight at 44°C, and subsequently streaked onto violet red bile agar and incubated for 24h at 44°C by RFCA. Presumptive *E. coli* were further identified by CHROMagar Orientation Medium (one laboratory) or by indole- and lactose testing in laurylsulphate-broth culture incubated overnight at 44°C. *E. coli* isolates were sent to the National Food Institute for MIC-testing. All isolates were stored (-80°C).

Enterococci were isolated by adding 5 g of the sample to 45 ml of azide dextrose broth, which was incubated overnight at 44°C and subsequently streaked onto Slanetz-Bartley agar. After incubation at 44°C for 48 hours, colonies typical of *E. faecium* and *E. faecalis* were further identified by the following criteria: Colour of material, motility, arginine dihydrolase testing and the ability to ferment mannitol, sorbitol, arabinose, raffinose and melibiose. All isolates of *E. faecium* and *E. faecalis* were stored (-80°C) and sent to the National Food Institute for MIC-testing.

4.3. Humans

Salmonella. isolates were serotyped according to the Kauffman-White Scheme.

Campylobacter. Species identification was performed using a species specific PCR assay [Klena *et al.* 2004, J Clin Microbiol. 42: 5549-5557].

Staphylococcus aureus. Sequencing of the *S. aureus* specific *spa* gene was used both for species conformation and typing purposes. Any *spa* negative isolates were confirmed as *S. aureus* by MALDI-TOF. The spa typing [Harmsen *et al.* 2003. J Clin Microbiol. 41: 5442-5448] and additional typing by multi locus sequence typing (MLST) was performed [Enright *et al.* 2000. J Clin Microbiol. 38: 1008-1015] and annotated using eBURST v.3 software (www.mlst.net). Based on the *spa* and MLST typing, each isolate was assigned to a clonal complex (CC). For MRSA isolates, presence of the *mecA* methicillin resistance gene was confirmed by PCR [Larsen *et al.* 2008. Clin Microbiol Infect. 14: 611-614].

5. Susceptibility testing

MIC-testing
Antimicrobial susceptibility testing of ***Salmonella, Campylobacter,* indicator *E. coli, Enterococcus*** and the veterinary pathogens was performed as microbroth dilution MIC with the Sensititre system (Trek Diagnostic Systems Ltd., East Grinstead, United Kingdom). Inoculation and incubation procedures were in accordance with the CLSI guidelines. The following quality control strains were used for internal control: *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212 and *Campylobacter jejuni* ATCC 33560.

Table AP2.2 presents an overview of the interpretation of MIC-values used for all combinations of bacteria and antimicrobial agents. Since 2007, MIC-data were interpreted using EUCAST epidemiological cut-off values with a few exceptions as explained by footnotes in Table AP2.2. In addition, corresponding clinical breakpoints are also presented in Table AP2.2 and shown in all MIC-distributions in order to visualize the impact of using epidemiological cut-off values contra clinical breakpoints. In most cases, data from previous years presented in this DANMAP report are not corrected for changes in interpretation (e.g. all data are presented with use of the interpretation applied for the year in question).

All isolates from animals and meat were MIC-tested at the National Food Institute. The *Salmonella* and *Campylobacter* isolates of human origin were tested at SSI. From 1998–2007, a performance test for susceptibility testing was carried out once a year to ascertain the quality and comparability of susceptibility testing in the laboratories providing MIC-data. In 2002, MIC-testing at the National Food Institute was accredited by DANAK (the Danish national body for accreditation), and the SSI is awaiting the same accreditation. Both laboratories participate in European ring trials to ensure MIC-data of constantly high quality.

Table AP2.2. EUCAST epidemiological cut-off values (blue fields) and corresponding clinical breakpoints used as interpretation criteriae for MIC-determination

DANMAP 2010

Antimicrobial agent	*Salmonella*		*E. coli*		*E. faecium*		*E. faecalis*		*C. jejuni*		*C. coli*	
	Epid cut-off µg/ml	Clin break µg/ml	**Epid cut-off µg/ml**	Clin break µg/ml	**Epid cut-off µg/ml**	Clin break µg/ml	**Epid cut-off µg/ml**	Clin break µg/ml	**Epid cut-off µg/ml**	Clin break µg/ml	**Epid cut-off µg/ml**	Clin break µg/ml
Ampicillin	**>8***	>8*	**>8***	>8*	**>4***	>8*	**>4***	>8*				
Apramycin	**>16**		**>16**									
Avilamycin					**>16***		**>8***					
Cefotaxime	**>0.5***	>2*	**>0.25***	>2*								
Cefoxitin												
Ceftiofur	**>2***		**>1***									
Chloramphenicol	**>16***	>16	**>16***	>16	**>32***	>16	**>32***	>16	**>16***		**>16***	
Ciprofloxacin	**>0.06 ***	>1*	**>0.03***	>1*	**>16 b)**		**>8 b)**		**>1***	>1*	**>1***	>1*
Colistin	**>2***	>2*	**>2***	>2*								
Erythromycin					**>4***	>4	**>4***	>4	**>4***	>4*	**>16***	>16
Florfenicol	**>16***		**>16***									
Gentamicin	**>2***	>4*	**>2***	>4*	**>32***	>512	**>32***	>512	**>1***		**>2***	
Kanamycin					**>1,024**		**>1,024**					
Linezolid					**>4***	>4*	**>4***	>4*				
Nalidixic acid	**>16***	>16	**>16***	>16					**>16***		**>32***	
Neomycin	**>4***		**>8***									
Penicillin					**>16***	>8	**>16***	>8				
Quinupristin/ dalfopristin					**>4 a)**	>4*						
Salinomycin					**>4**		**>4**					
Spectinomycin	**>64**		**>64***									
Streptomycin	**>16***		**>16***		**>128***		**>512***		**>2***		**>4***	
Sulfonamide	**>256**	>256	**>256**	>256								
Teicoplanin					**>2***	>2*	**>2***	>2*				
Tetracycline	**>8***	>8	**>8***	>8	**>4***	>8	**>4***	>8	**>2***	>8	**>2***	>8
Tiamulin												
Tigecycline					**>0.25***	>0.5*	**>0.25***	>0.5*				
Trimethoprim	**>2***	>4*	**>2***	>4*								
Vancomycin					**>4***	>4*	**>4***	>4*				

* EUCAST epidemiological cut-off value or EUCAST clinical breakpoint. Clinical breakpoints defined by CLSI (unmarked) were listed if a clinical breakpoint was not defined by EUCAST

a) The EUCAST epid. value of >1 was not applied according to investigations presented in DANMAP 2006 (trade name synercid)

b) The EUCAST epid. value of >4 was not applied, since the purpose was to look for high level ciprofloxacin resistance as described by Werner *et al.* 2010 (Int J Antimicob Agents. 35: 119-125)

One isolate per bacterial species per farm, per meat sample, or per patient was tested for antimicrobial susceptibility. For animal isolates in excess numbers (typically for indicator *E. coli*, enterococci and *Campylobacter* from healthy production animals), a random selection of 100 to 150 isolates was appointed to MIC-testing. Due to low number of isolates, *C. jejuni* from pigs, *C. coli* from cattle, *C. coli* from broilers, and *S. hyicus* from pigs (clinical cases) were not susceptibility tested.

***Staphylococcus aureus* from humans**

Susceptibility testing was performed by disc diffusion according to EUCAST methodology using discs from Oxoid (Ballerup, Denmark) on Mueller-Hinton Agar (SSI, Copenhagen, Denmark). The following antimicrobials were tested: Erythromycin, clindamycin, kanamycin, rifampicin, penicillin, cefoxitin, fusidic acid, norfloxacin, linezolid, tetracycline and mupirocin. In addition, MRSA isolates were screened for susceptibility towards glycopeptides by spot test on Brain-Heart infusion (BHI) agar (Becton Dickinson, Germany) with teicoplanin (5 mg/L) and confirmed by Etest® (AB Biodisk, Solna, Sweden) on BHI with inoculum of McFarland 2.0. In case of MIC ≥ 8 mg/L for vancomycin and teicoplanin or an MIC ≥12 mg/L for teicoplanin, population analysis profile against vancomycin was performed [Wootton *et al.* 2001. J Antimicrob Chemother. 47: 399-403].

Invasive *Streptococcus pneumonia* from humans

Screening for penicillin-resistant *S. pneumoniae* was performed using a 1 µg oxacillin tablet (Neo-Sensitabs®, A/S Rosco, Taastrup, Denmark) on 10% horse blood agar (SSI Diagnostika, Hillerød, Denmark), and for erythromycin-resistant *S. pneumoniae* using a 78 µg erythromycin tablet (Neo-Sensitabs®, A/S Rosco, Taastrup, Denmark) on 10% horse blood agar (SSI

Diagnostika). The breakpoints used are defined by the CLSI. Penicillin and erythromycin MICs were determined using STP6F plate, Sensititre (Trek Diagnostic Systems Ltd., East Grinstead, United Kingdom) as recommended by the manufacturer. The breakpoints used are defined by EUCAST. Resistant isolates were defined as both fully and intermediary resistant isolates.

Invasive *Streptococcus pyogenes* (group A), group B, C and G streptococci from humans
Screening for penicillin-resistant streptococci was performed using a 1 µg oxacillin disk (Oxoid, Greve, Denmark) on 10% horse blood agar (SSI Diagnostika, Hillerød, Denmark), and for erythromycin-resistant streptococci using a 78 µg erythromycin tablet (Neo-Sensitabs®, A/S Rosco, Taastrup, Denmark) on 10% horse blood agar (SSI Diagnostika). Erythromycin resistant streptococci were tested with a 15 µg erythromycin disk (Oxoid) and a 2 µg clindamycin disk (Oxoid) on Mueller-Hinton Agar (Mueller-Hinton plate, 5% blood, 20 mg beta-NAD, SSI Diagnostika). Erythromycin MICs were determined using the Etest® (AB Biodisk, Solna, Sweden) on Mueller-Hinton incubated at 36°C, 5% CO_2. The breakpoints used are defined by the EUCAST. Resistant isolates were defined as both fully and intermediary resistant isolates.

***E. coli, K. pneumoniae, P. aeruginosa*, invasive *E. faecium* and *E. faecalis* from humans**
In 2010, the DCM at hospitals in Næstved, Odense and Viborg, and Rigshospitalet, that is the national referral hospital, used the tablet diffusion method (Neo-Sensitabs®, A/S Rosco) on Danish Blood Agar (Resistensplade, SSI Diagnostika) and the breakpoints defined for this medium by A/S Rosco.

However, the DCM at Odense Hospital used Neo-Sensitabs® on Mueller-Hinton agar (SSI Diagnostica) when testing urine isolates and Columbia agar with 4.5% NaCl (SSI Diagnostika) when testing staphylococci for oxacillin resistance. The DCM at Vejle Hospital used the Neo-Sensitabs® on Mueller-Hinton agar (SSI Diagnostica) and the breakpoints defined for this medium by A/S Rosco. The DCM at Esbjerg Hospital used the tablet diffusion method (Neo-Sensitabs®, A/S Rosco) on Mueller-Hinton agar (SSI Diagnostika) when testing *E. coli.* The DCM at Aalborg Hospital used the Neo-Sensitabs® on Mueller-Hinton agar (SSI Diagnostika) in combination with the tablet diffusion method (A/S Rosco) and the breakpoints defined by the Swedish Reference Group for Antibiotics (SRGA) (available from: URL: http://www.srga.org/). The only exception from SRGA was that the wild type population of *E. coli* was deemed susceptible for ampicillin instead of intermediary susceptible.

In 2010, the DCM at Hillerød and Hvidovre Hospitals used the disk diffusion method (Oxoid, Basingstoke, UK) on Iso-Sensitest (ISA) medium (Oxoid). The DCM at Slagelse Hospital used the same disks on Iso-Sensitest (ISA) medium with or without 5% horse blood (Oxoid) according to test material and bacterial species. Laboratories performing the disk diffusion method used the breakpoints defined by the SRGA. In 2010, the DCM at Herlev, Herning and Aarhus Hospitals changed to methods described by EUCAST (DCM at Aarhus and Herning hospitals per February and May 2010, respectively).

All submitting laboratories participate in national and international quality assurance collaborations such as the United Kingdom National External Quality Assessment Schemes (NEQAS).

Quinupristin/dalfopristin breakpoint
The epidemiological cut-off value suggested by EUCAST for quinupristin/dalfopristin when testing *E. faecium* is >1 µg/ml. In DANMAP, *E. faecium* isolates with MICs >4 µg/ml are reported resistant to quinupristin/dalfopristin due to an evaluation study presented in the DANMAP 2006 report, page 49-50.

6. Data handling

6.1. Animal

The results from the primary examination of samples from slaughterhouses and primary production for the bacteria of interest - positive as well as negative findings - and of the susceptibility testing were stored in an Oracle Database 8i Enterprise Edition® at the National Food Institute. The susceptibility data were stored as continuous values (MIC) as well as categorised as susceptible or resistant, respectively, as defined by the relevant epidemiological cut-off value. Each isolate was identified by the bacterial species, including subtype as applicable and by the date of sampling and the species of animal. Information on the farm of origin was also recorded. All handling and evaluation of results was carried out using SAS®Software, SAS Enterprise Guide 3.0.

6.2. Meat

Results from the analysis of food samples were reported via the database administrated by the Danish Veterinary and Food Administration, except for the data on *Salmonella*, which were reported to and extracted from the laboratory database at the National Food Institute. For each bacterial isolate, information was available on the food type, bacterial species, date and place of sampling, date of examination of the sample, country of slaughter, the RFCA that collected and processed the sample, and an identification number, which makes it possible to obtain further information about the isolate from the Authority. Furthermore, more detailed information about the country of origin was recorded whenever possible.

6.3. Human

Salmonella* and *Campylobacter. Data on *Salmonella* and *Campylobacter* infections are stored in the Danish Registry of Enteric Pathogens (SQL database) maintained by SSI. This register includes only one isolate per patient within a window of six months and

includes data on susceptibility testing of gastrointestinal pathogens.

Staphylococcus aureus. For MRSA, data on the characteristics of the isolates and the clinical/epidemiological information were obtained from the Danish MRSA register at SSI (mandatory reportable). In this database, patients were only registered the first time they were diagnosed with MRSA regardless of whether it was colonisation or infection. Based on the reported information, MRSA cases were classified as colonisation/active screening (i.e. surveillance samples to detect nasal, throat, gut or skin colonisation), imported infection (i.e. acquired outside Denmark), infection acquired in a Danish hospital, defined as diagnosed >48 hours after hospitalisation with no sign of infection at admittance (HA-MRSA) or infection diagnosed outside hospitals (community onset).

MRSA cases with community onset were further classified according to risk factors during the previous 12 months as either health-care associated with community onset (HACO) or community-acquired (CA). Health-care associated risk factors included prior hospitalisations or stay in long-term care facilities within 12 months prior to MRSA isolation and being a health-care worker. Community risk factors included known MRSA positive household members or other close contacts. Non-Danish origin defined as the person or one of the parents being born outside Denmark was investigated through the Danish Civil Registry.

Streptococcus pneumoniae, Streptococcus pyogenes **(group A streptococci), group B, C and G streptococci**. Data on susceptibility testing of isolates were stored as MICs in a Microsoft® Access database placed at a SQL server at SSI. Analysis including selection of isolates from blood and spinal fluid samples and removal of duplicate isolates was performed in Microsoft® Access.

E. coli, K. pneumoniae, P. aeruginosa, **invasive *E. faecium* and invasive *E. faecalis.*** Fourteen DCM provided data on resistance levels in *E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*, invasive *E. faecium* and invasive *E. faecalis* isolates. Data were extracted from the following laboratory information systems:
- ADBakt (Autonik AB, Skoldinge, Sweden) for the DCM at Hvidovre, Herlev and Aalborg Hospitals.
- MADS (DCM, Skejby Hospital, Aarhus, Denmark) for the DCM at Rigshospitalet and Slagelse, Næstved, Roskilde, Odense, Esbjerg, Vejle, Herning, Aarhus (Skejby) and Viborg Hospitals.
- SafirLIS Microbiology (Profdoc Lab AB, Borlänge, Sweden) for the DCM at Hillerød Hospital.

Resistance data on the first isolate per patient per year were included. Generally, resistance data were excluded if susceptibility to a certain antimicrobial agent was tested on only a selected number of isolates.

7. Calculation of confidence limits

Estimation of exact 95% (two-sided) confidence intervals for proportions were based on binomial probability distributions as described in Armitage & Berry [Statistical Methods in Medical Research, 4th ed. 2001, Oxford: Blackwell Scientific Publications]. Significance tests of differences between proportions of resistant isolates were calculated using SAS®Software, SAS Enterprise Guide 3.0 or StatCalc in EpiInfo™ v. 6. In the text, significant differences imply statistically significant differences where $p<0.05$ using Chi-square, or Fisher's Exact Test when the number of samples is low (<25).

Anne Mette Seyfarth (animal and meat data) and Line Skjøt-Rasmussen (human data)

APPENDIX 3

Danmap Publications 2010

Aarestrup FM, Cavaco L, Hasman H. Decreased susceptibility to zinc chloride is associated with methicillin resistant *Staphylococcus aureus* CC398 in Danish swine. Vet Microbiol. 2010 May 19;142(3-4):455-7.

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Aarestrup FM, Skov RL. Evaluation of ceftiofur and cefquinome for phenotypic detection of methicillin resistance in *Staphylococcus aureus* using disk diffusion testing and MIC-determinations. Vet Microbiol. 2010 Jan 6;140(1-2):176-9.

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